State of the Art Review on the Uses of Microorganisms in Mineral Benefication and Ore Processing.

FINAL REPORT
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Summary

This report is an in depth survey of the literature relating to the use of microorganisms in mineral ore processing and beneficiation. Included in this are the processes of leaching, adsorption and precipitation. These processes are specifically concerned with the processing of metal-containing ores. The recovery of oils and the desulphurization of coal is also covered briefly.

A great deal of investigation in the laboratory has been carried out on the leaching process. This involves the use of microorganisms which oxidize metal sulphides to sulphates. These sulphates become soluble and consequently the metal is released. Leaching trials have been carried out on copper, uranium, cadmium, aluminum, lead, zinc, nickel, gold, silver, vanadium, and arsenic. The efficiency of leaching the various metals is dependent on a number of factors including temperature. pH, surface area of the ore, and inorganic nutrients. In addition, the mineral ore cannot contain much carbonate otherwise the solution becomes too basic. Most studies on the leaching process have been carried out in the laboratory using the bacterium Thiobacillus ferrooxidans although other bacterial species have been investigated. Some pilot and full scale studies have been carried out but none of them have been successful commercially. The usual commercial process is dump leaching which is inexpensive but very slow and inefficient.

Studies on the desulphurization of coal have shown that the same organisms used in leaching metals can also be used to upgrade coal by removing sulphides responsible for sulphur dioxide production on combustion.

Processes to recover ores after they have been solubilized have been investigated in the laboratory only. Adsorption by binding or through intracellular uptake has been studied in yeast and bacteria. It has been found to occur with

copper, lead, selenium, molybdenum, iron and uranium as well as precious metals and nickel, zinc, aluminum, and tin and there are many species of microorganisms in which it occurs. The uptake is affected by temperature, pH, and the presence of other ions and amino acids. Two patents have been issued to use fungal mycelium from various fungi reinforced in such a way that they can retain uranium, radium and lead. A patent using numerous species of fungi also has been granted to extract numerous metals from acidic solution.

A continuous process to recover copper, zinc and iron from a bacterial leaching solution has been reported. It has been found that a species of Penicillium concentrated and accumulated copper from weak solutions containing metal ions. Negatively charged polysaccharides excreted by microorganisms have been found to react with metal ions in solution and produce insoluble precipitates in which the metals are trapped.

Bacterial and yeast cells can be immobilized in polyacrylamide gels and then used to accumulate metal ions. The metal ions can be recovered by chemical treatment, concentrated and recovered.

Résumé

Ce rapport est une recherche intensive de la littérature ayant rapport à l'utilisation de micro-organismes dans le traitement et l'enrichissement de minerais. Compris dans cela sont les processus de lixiviation, d'adsorption et de précipitation. Ces processus s'occupent aux traitements des minerais qui contiennent des métaux. Le recouvrement de pétroles et la désulfuration de charbon est inclut brièvement.

Beaucoup de recherche a été faite dans des laboratoires du processus de lixiviation. Cela inclut l'utilisation des micro-organismes qui oxydant les métaux sulfures aux métaux sulfates. Ces sulfates deviennent solubles et par conséquent le métal est dégagé. Les expériences de lixiviation ont été faites sur le cuivre, l'uranium, le cadmium, l'aluminium, le plomb, le zinc, le nickel, l'or, l'argent, le vanadium et l'arsenic. L'efficacité pour lixivier les métaux variés est dépendante d'un nombre de facteurs, la température, le pH, la surface du minerai et les nutritives inorganiques. De plus, les minerais ne devraient pas contenir trop de carbonate autrement la solution deviendra trop basique. La plupart des études du processus de lixiviation ont été faites dans des laboratoires en utilisant la bactérie Thiobacillus ferrooxidans mais d'autres espèces de bactéries ont été étudiées aussi. Des projets pilotes et de grandes opérations ont été exécutés mais ils n'ont pas réussit commercialement. Le processus commerciel courant est la lixiviation en tas (heap lixiviation) qui est peu coûteux mais qui est très lent et inefficace.

Les recherches de la désulfuration de charbon ont montré que les mêmes organismes utilisés dans la lixiviation des métaux peuvent être utilisés aussi pour améliorer la qualité de charbon en enlevant les sulfures qui produit bioxyde soufre en combustion.

Les cellules bactériennes et les cellules de levures peuvent être immobilisées dans des gels polyacrylamide et ensuite elles peuvent être utilisées pour accumuler les ions de métaux. Les ions des métaux peuvent être recouvrés par un traitement chemique. Ils peuvent être concentrés et recouverts.

Les processus pour recouvrer les minerais après qu'ils soient solubilisés ont été recherchés seulement dans des laboratoires. On a étudié l'adsorption sur les bactéries et l'adsorption sur les levures. Il y a eu l'adsorption du cuivre, du plomb, du sélénium, du molybdène, du fer, de l'uranium, du nickel, du zinc, de l'aluminium, de l'etain et des métaux précieux aussi. Il y a beaucoup d'espèces de micro-organismes où cela se trouvent. L'adsorption est touchée par la température, le pH, la presénce d'autres ions d'acides-aminés. Deux brevets permettent l'utilisation de mycélium des fongi variés renforcés dans une manière où ils peuvent garder l'uranium, le radium et le plomb. Un brevet utilisant beaucoup de genres de fongi a été accordé pour l'extraction de plusieurs métaux d'une solution acide.

On a rapporté un processus continuant pour recouvrer le cuivre zinc de fer d'une solution de lixiviation bactérienne. On a trouvé qu'une espèce de Penicillium a concentré et a accumulé le cuivre d'une solution faible contenant des ions de métal. Des polysaccharides chargées négativement excretées par des micro-organismes ont réagit avec des ions de métal dans une solution et cela a produit des précipités insolubles.

Research Recommendations

Although it was not the purpose of this study to investigate the economic feasibility of the various biological beneficiation processes available, this question was addressed in the literature search. Few publications were unearthed dealing with this question. Since the research on leaching has been undertaken for a long period of time, it should have been at the point where economic feasibility and cost sensitivity analysis had been investigated in depth. Numerous instances of pilot plant and full scale operating systems are documented in the literature.

As the demand for new minerals increases and high grade ores become depleted, the mining industry will become increasingly dependent on the exploitation of lower grade material. In addition to this, the ever increasing concern over the environmental effects of acidic gaseous products causing acidic rain may mean that less environmentally harmful processes have to be adapted. Although at the present time biological leaching appears to be more costly than the conventional smelting processes, it may be that with more intensive studies of leaching and other biological recovery processes, new processes may emerge which can compete favourably with the expensively upgraded conventional processes modified to minimize emissions harmful to the environment.

A new look has to be taken into each aspect of the biological processes presented in this review to determine where the improvements and efficiencies can best be gained. One area which applies to all biological processes which may have commercial benefit is that mixed discipline groups involving geologists, microbiologists, chemists, geneticists, mining engineers, biochemical engineers, and economists should be involved jointly to decide where the effort for the most return should be put. In the bacterial leaching area, the chemistry is quite well understood; however, the microbial aspects need a greater understanding. There are many conflicting results published on the metabolism and other parameters required to bring about efficient leaching. It could well be that investigators are not using the same organisms as their colleagues even though they name the organisms and describe them the same. Genetic engineering principles could be applied to bring about increased leaching efficiencies at lower temperatures. In the Canadian climate conventional leaching proceeds too slowly for most of the year to make in situ leaching a practical process. If pyschophilic properties could be engineered into the leaching bacteria then the process could be operated in the colder time of the year. While thermophilic organisms would be more productive when active, the short Canadian summers would preclude the development of thermophilic or even mesophilic strains.

The bacterial leaching process is no different than any other biological process and with the proper engineering designs for maximum efficiency in the areas of mass transfer of oxygen, nutrient requirements and substrate concentration (such has been done with activated sludge sewage plants) coupled with production of an organism tailored for the job by genetic manipulation, there is no reason why an efficient leaching process cannot be developed. The greatest problem to be forseen is one that may be insurmountable and that is the intrinsic properties of the ore. It is already known that some mineral deposits are not amenable to bacterial leaching by present techniques.

In situ leaching, while it appears to be inexpensive, is not necessarily the method of choice. The conditions cannot be controlled and unpredictable results are inevitable. The only sure thing is that leaching will occur but yields and rates of leaching will always be unpredictable.

By and large the state of knowledge of leaching is at the stage where pilot plant studies are necessary to put laboratory findings into practice. Further laboratory studies should not he undertaken until pilot studies have pointed out the gaps in the knowledge. Once the gaps in knowledge have been identified, then laboratory studies should be implemented to solve problems caused by voids in knowledge.

The need in leaching is to produce organisms resistant to high metal concentrations that can oxidize specific metal sulphides and that are operative at a low temperatures.

Processes to remove metals in solution using microbial cultures are at the present time little more than laboratory curiosities although some practical applications of the process has been studied in an attempt to remove heavy metals from sewage plant effluents.

Biological adsorption of metals from solution is an area where more attention should be focussed. This phenomenom rather than biological leaching can have far reaching implications for the reduction of emmissions of oxides of sulphur from metal smelters. It is foreseeable that organisms genetically tailored can be used to selectively remove metals from solution. Organisms can handle much lower concentrations of metals than it is economically feasible to handle by conventional methods.

The technical feasibility of these processes has been demonstrated in the laboratory. Further investigations to demonstrate the problems inherent in commercializing these techniques are necessary.

SECTION I

BIOLOGICAL LEACHING

BIOLOGICAL LEACHING

Bacterial leaching involves the oxidation of metal sulphides to sulphates by the following generalized reactions:

$$MS + 2O_2 \xrightarrow{\text{bacteria}} MSO_4$$

$$MS \longleftrightarrow M^{++} + S^{-}$$

$$S^{-} + 2O_2 \xrightarrow{\text{bacteria}} SO_4^{--}$$

M represents a bivalent heavy metal such as Cu, Ni, Zn, etc. The metal sulphides are insoluble but the sulphates are soluble in the acidic leach solution.

Microorganisms that can release soluble forms of metals from insoluble ores in an acidic medium are of great industrial significance. The chemolithotropic Thiobacillus group are particularly important in this phenomenon. For example, Thiobacillus ferrooxidans derives its energy for metabolic processes from the oxidation of ferrous iron, metal sulphides and soluble sulphur compounds in an acidic medium.

Bacteria provide the required oxidizing condition in a leach solution either directly or indirectly. Direct bacterial leaching involves an enzymatic attack by bacteria on components of the mineral that can be oxidized. For example, microorganisms such as Thiobacillus ferrooxidans, TH thiobacilli and Sulfolobus can mobilize copper by directly oxidizing a metal-sulphide substrate (Brierley, J.A., 1981).

e.g. $Cu_2S + H_2SO_4 + 2\frac{1}{2}O_2 \longrightarrow 2CuSO_4 + H_2O$

The oxidized product is more water-soluble. Although there has been considerable controversy as to whether or not this mechanism exists, several researchers have provided evidence to support this theory (Dave et al., 1979b; Gaidarjiev et al., 1975; Silverman, 1967; Torma, 1978; Touvinen and Dispirito, 1984).

Indirect or bacterially-assisted leaching is dependent on the oxidizing action of ferric ions. Bacterial oxidization of ferrous iron (Fe^{++}) results in the formation of ferric iron (Fe^{+++}) and sulphuric acid. The ferric iron acts as an oxidizing agent which reacts with metals to form oxidized products that are more soluble in a sulphuric acid solution. During this process, the ferric iron is reduced to the ferrous state. The bacteria regenerate ferric iron to complete the cycle. The microbial activity can be separated by space and time from the actual metal solubilization process (Brierley, J.A., 1981; Tuovinen et al., 1983).

Brierley (J.A., 1981) proposed that "the leaching bacteria may also participate in an electrochemical process of mineral oxidation. The metal sulphide acts as an anode (1) and the cathode reaction occurs with either oxygen (2), ferric iron (3), or a mineral of more positive electrode potential.

$$(1)\ CuS \longrightarrow S + Cu^{2+} + 2e$$

$$(2)\ O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$$

$$(3)\ Fe^{3+} + e^- \longrightarrow Fe^{2+}$$

The microorganisms can act as catalysts for the transfer of electrons to oxygen (2), or the generation of ferric iron (3) which serves as the anode. Microbes may also participate in the electrochemical leaching process by removal of sulphur formed during the oxidation step (1) ".

$$S + H_2O + 1\tfrac{1}{2}\ O_2 \longrightarrow H_2SO_4\ (4)$$

Bacterial leaching is confined to the treatment of ore materials that do not contain excessive amounts of carbonate minerals because most metals are insoluble in alkaline solutions and the Thiobacillus ferrooxidans is an obligate acidophile. The carbonate minerals can be neutralized by continuously adding sulphuric acid to the leach material but insoluble precipitates (eg. gypsum) may form on the ore surfaces and the reagent consumption may increase (Tuovinen and Dispirito, 1984).

PARAMETERS AFFECTING BACTERIAL LEACHING

In any oxidation system there are optimum operating conditions. Environmental conditions are more restricted in a biological system than in a chemical process because a biological system involves living organisms. Lundgren and Silver (1980) listed the parameters that can affect bacterial leaching:

1. Temperature
2. pH
3. Oxidation-reduction potential (Eh) - a measure of the tendency of a substance to accept or denote electrons and is used to measure the oxidizing ability of a given environment.
4. Composition of the leaching medium - many growth media have been described and used in laboratory studies but are too expensive to use in large scale mining operations. Nutrients must be identified which are needed to add to a leaching solution.

An organism can fulfill its requirement for sulphur and an acidic environment with sulphate. Sulphate is a product of sulphide mineral oxidation and a by-product of ferrous iron oxidation. Potassium, calcium and magnesium are present in leaching solutions in excess of the minimal requirements for growth. Phosphate and ammonia are the only growth requirements that are generally not present in leach solutions.

Phosphate may be supplied when the phosphate-containing mineral apatite is present in the ores. Blasting and milling operations may result in residual amounts of ammonia present in the leaching solution.

5. Particle size and substrate concentration (pulp density)

ADVANTAGES OF MICROBIAL LEACHING

An important aspect of microbial leaching is selectivity, that is, different metals can be removed separately from the same ore.

Microbial leaching methods are more economical than conventional methods especially with low-grade ores and inaccessible material from mining operations where conventional methods are uneconomical (Gupta and Sant, 1970). Valuable metals and minerals from old mines and dumps can be extracted. The transportation and smelting of ores are eliminated. Equipment and other capital costs are small and the energy requirements are limited to the pumping of solutions. There is no air pollution associated with the process.

DISADVANTAGE OF MICROBIAL LEACHING

Bacterial leaching takes longer than the conventional leaching methods.

COMMERCIAL LEACHING METHODS

Numerous reviews which discuss leaching practices have been published (Brierley, C.L., 1978b; Bruynesteyn and Duncan, 1971, 1976; Cathles and Apps, 1975; Fletcher, 1970; Lundgren and Silver, 1980; Stirkov et al., 1975; Wadsworth, 1981). Dump leaching and heap leaching are the two most prevalent commerical leaching methods used in the bacterial extraction of mineral ores (Lundgren and Malouf, 1983). Dump leaching is a process in which hundreds of tons of low-grade ores or waste rock are deposited in valleys or on other impermeable ground. There is usually no preparation of material size or segregation of different types of materials prior to the addition of the leaching solution. The leaching solution can be introduced naturally or by spraying, flooding or injecting through vertical pipes. This solution percolates to the bottom of the pile of ore and is collected. The metals can then be concentrated from this solution. Heap leaching is a similar leaching method but finer and more concentrated ores are used. The ores are placed on prepared drainage pads instead of on the naturally occurring terrain.

Most leaching dumps are situated on the existing topography of the area. The dumps are located on impermeable ground when possible, with a drainage system that enables easy collection of the leach solution.

IN SITU LEACHING TECHNIQUES

Mined ores may be brought to the surface before it is found that they are too low in metal values to be worth hauling to the plant for processing. In situ leaching concentrates metal values in liquors which can be piped, pumped or hauled to a plant for recovery. Technology used in in situ leaching is:

1) Heap leaching
2) Hillside heap leaching
3) Hole-to-hole leaching
4) Hole-to-mine leaching
5) Routine spray leaching of stopes and mined-out areas

Zajic (1969) suggested that the leaching of elements by any of the above procedures may be limited by:

1. Availability of cheap water
2. Type of ore - ore must contain elements that can be oxidized by microbes to form oxidized metals (Fe^{3+}, Mn^{2+}) which can oxidize or solubilize the desired metal or mineral sulphides which can be oxidized by microorganisms to yield ferric sulphate and sulphuric acid.
3. Growth of a suitable microorganism.
4. Availability of other cheap chemical additives which aid in leaching.
5. Cheap solvent extraction of the desired element from dilute aqueous solutions.

LABORATORY PROCEDURES FOR INVESTIGATING LEACHING

Shake flask tests use small amounts of finely ground ore to test if an ore is amenable to bacterial leaching. Maximum obtainable extraction; maximum rate of extraction; the amount of acid consumed/produced during the leach; and the amount of extraction resulting from the sulphuric acid content of the leach are evaluated by these laboratory experiments (Anon, 1979; Lundgren and Silver, 1980). If the ore is amenable to leaching, column leaching tests are performed to simulate heap and dump leaching conditions (Silver and Lundgren, 1980). Column leaching tests are used to reveal the effects of particle size, clay formations and mineral distribution on the rate and extent of mineral extraction (Anon, 1979). The maximum grade of pregnant solution for a given height; changes in the rate of extraction; and the optimum dump heap configurations can be determined by the column tests to further determine the feasibility of leaching the ore in a commercial operation.

Moss and Anderson (1968) outlined the advantages and limitations of using shake flasks, leach columns, batch reactors and continuous culture for investigating the leaching phenomenon.

A REACTOR SYSTEM FOR MINERAL LEACHING INVESTIGATIONS

Babij et al. (1980) describe a reactor system for assessing the suitability of a mineral-containing material for bacterial leaching. The researchers feel that the system ensures proper suspension of ore particles, minimizes the abrasive effect of ore particles, provides adequate aeration and ensures the recovery of the leach residues. Mineral biodegradation can be studied under a wide range of physiochemical conditions and with numerous types of microorganisms. The system may be used in the determination of the feasibility of the recovery of copper, zinc, cobalt and uranium from low-grade ores, for the beneficiation of gold-bearing, tin-containing or siliceous materials and for the assessment of environmental hazards caused by overburden heaps and tailings dams.

Murr et al. (1977) outlined the design criteria and sampling methods for large scale leach tests on low grade copper waste. The temperature profiles and chemical and biological activity in a 1.63×10^5 kg waste sample were discussed.

USE OF A "CYTOPHEROMETER" IN ELECTROPHORETIC STUDIES OF MINERAL-LEACHING BACTERIA

Ahmed (1971) modified a "Cytopherometer" by incorporating palladium electrodes with electrochemically occluded hydrogen to conduct electrokinetic studies of mineral suspensions and of mineral-leaching bacteria. The Cytopherometer enabled fast and accurate electrokinetic measurements to be made on mineral suspensions in liquids. Electrokinetic studies with mineral-leaching bacteria (Thiobacillus ferrooxidans) were carried out in neutral and acidic solutions. The bacteria did not exhibit any surface change in these solutions which was attributed to a protective cell membrane which isolated the bacterial cell from the solution. In alkaline solutions ($pH>9$), the bacteria developed a strong negative surface charge. This was attributed to the rupture of the protective membrane and the formation of $R - NH_2 - COO^-$ groups on the cellular surface. Ahmed (1971) suggested that any ionic surface-active agent that is used to accelerate the leaching of minerals should have a charge opposite to that of the mineral surface. The non-ionic group of the surfactant would adhere to the bacteria.

SECTION I.1

MICROORGANISMS INVOLVED IN LEACHING

MICROORGANISMS INVOLVED IN LEACHING

IRON-OXIDIZING THIOBACILLI

The iron-oxidizing thiobacilli are unique amongst thiobacilli as this group of bacteria can oxidize ferrous iron for the generation of chemical energy. Metal sulphide ores can also be oxidized to provide energy for the microorganism. This oxidation changes the sulphide moiety to sulphate and modifies the structure of the initial substrate. As a result, metallic constituents of the sulphide ores may be solubilized.

The metabolism of the thiobacilli has been reviewed by McCready (1976).

THIOBACILLUS FERROOXIDANS

Thiobacillus ferrooxidans cells are short rods which can occur singly or in pairs but rarely in short chains (Buchanan and Gibbons, 1974). This obligate aerobic bacterium depends upon ferrous iron as its sole energy source. The following reaction is catalyzed by the bacterium to obtain energy (Silverman and Lundgren, 1959):

$$1)\ 4FeSO_4 + 2H_2SO_4 + O_2 \longrightarrow 2Fe_2(SO_4)_3 + 2H_2O$$

Upon hydrolysis, the following reaction takes place which produces more acid than is used in the above reaction.

$$2)\ 2Fe_2(SO_4)_3 + 12H_2O \longrightarrow 4\ Fe(OH)_3 + 6H_2SO_4$$

The net effect of these two reactions is a decrease in the pH of the surrounding environment.

$$(1 + 2)\ 4FeSO_4 + O_2 + 10H_2O \longrightarrow 4Fe(OH)_3 + 4H_2SO_4$$

Thiobacillus ferrooxidans is an obligate chemoautotroph which means that it must transport reduced iron (ferrous iron) into the cell, oxidize it to the ferric state and transport the ferric iron out of the cell (Siverman and Lundgren, 1959).

$$Fe^{++} \longrightarrow Fe^{+++} + e^{-}$$

The energy yield of the above reaction is low (11.3 Kcal/g-atom) and therefore large amounts of iron must be processed in order to meet the energy requirements of the cell. The bacterium utilizes ammonia as a nitrogen source; nitrates are used slowly. It is capable of oxidizing sulphur and other partially reduced sulphur compounds in addition to thiosulphate (Buchanan and Gibbons, 1974).

The temperature range for growth is between 15 and 35°C. Growth occurs between pH 1.4-6.0; the optimum pH is between 2.5 and 5.8 (Buchanan and Gibbons, 1974).

Other names: Ferrobacillus ferrooxidans, Ferrobacillus sulfooxidans

Silver (1978) suggested that Thiobacillus ferrooxidans is not one distinct microorganism but a group of metabolically similar microbes.

THIOBACILLUS FERROOXIDANS (SPK 1)

A new copper-concentrate-adapted strain of Thiobacillus ferrooxidans, SPK 1, has been found to have a high copper-leaching capacity (Babij and Madgwick, 1983). In experiments on the leaching of a Bougainville chalcopyrite concentrate, 93% of the copper was solubilized in the presence of Thiobacillus ferrooxidans (SPK 1) whereas only 8% of the copper was released in sterile controls.

The standard strain of Thiobacillus ferrooxidans (CPB 1) liberated 40% of the copper in the concentrate. Although the rate of copper release for the SPK 1 strain (93 mg/day) was less than that of the standard CPB 1 strain (117 mg/day), the SPK 1 strain had a higher copper to iron release ratio (2.04) than the CPB 1 strain (1.12). In other words, the new SPK 1 strain released less soluble ferric iron during copper leaching than the standard strain. Babij and Madgwick (1983) suggested that recovery of the soluble copper would be more efficient when there was less soluble iron present in the product solutions.

They also felt that low ferric iron release would avoid the build-up of secondary iron products on sulphide surfaces that have been found to inhibit bacterial action (Torma et al., 1979). For these reasons, SPK 1 may have a better potential for commercial use than CPB 1.

PRODUCTION OF THIOBACILLUS FERROOXIDANS

Silverman and Lundgren (1959) developed a medium capable of supporting large numbers of physiologically active cells of Thiobacillus ferrooxidans. A harvesting procedure to secure these high cell suspensions containing minimal amounts of precipitated iron was also developed (Silverman and Lundgren, 1959). The harvesting efficiency was between 40 and 65 percent.

Mynatt (1983) patented an apparatus and a process for the production of bacteria suitable for the leaching of metallic ore. The bacteria were inoculated on a pitted plate and supplied with a nutrient substrate to promote growth. The bacterial growth above the surface of the plate was harvested by passing a blade over the plate to shear off and collect the growth to be transferred to the ore leaching sites. The bacteria within the pits of the pitted plate were left behind for continued bacterial growth. The described process and apparatus is suitable for the production of Thiobacillus ferrooxidans and Leptothrix.

KINETICS OF THE DEVELOPMENT OF THIOBACILLUS FERROOXIDANS IN BATCH, SEMI-CONTINUOUS AND CONTINUOUS CULTURE

The maximum growth rate of Thiobacillus ferrooxidans grown in batch culture was calculated to be 0.1/hr and the mean rate of ferrous iron oxidation to be 0.2 g/hr (Egamberdieva et al., 1976). A semicontinuous process produced a mean oxidation rate of 0.4g/lhr. The maximum rate was 0.57 g/lhr.

ENUMERATING THIOBACILLUS FERROOXIDANS

Gormely and Duncan (1974) developed a method for estimating Thiobacillus ferrooxidans populations as bacterial nitrogen in systems where inorganics may precipitate as ammoniojarosite. The bacterial nitrogen was "estimated as the difference between the total nitrogen determined by the Kjeldahl digestion-caustic distillation and the inorganic nitrogen determined by caustic distillation.

Using this method for enumerating bacterial numbers, Gormely and Duncan (1974) found that 65% of the bacteria were attached to the solids during the leaching of a zinc suphide concentrate and the cell density was estimated to be 1.9 x 10^{10} cells/ml. During the leaching of chalcopyrite, the cell concentration was estimated at 8.6 x 10^{9} cells/ml with approximately 95-96% of the bacteria attached to the solids.

PYRUVIC ACID INHIBITION OF THIOBACILLUS FERROOXIDANS

In studies conducted by Nerkar et al. (1977), 85% of the uranium from uranium ores was dissolved out within 7 days using Thiobacillus ferrooxidans. Pyruvic acid inhibited the leaching of the uranium ores by this bacterium. It also inhibited the growth of Thiobacillus ferrooxidans. In both cases, inhibition was reversed in the presence of a Rhodotorula yeast species.

The mechanism of pyruvic acid inhibition is not known. Nerkar et al. (1977) suggest that the enzyme systems responsible for iron oxidation are located in the membranes of the bacterial cells and are vulnerable to pyruvic acid inhibition. This inhibition is reversed in the presence of the yeast as the pyruvic acid was assimilated by the yeast.

EFFECT OF FLOTATION AGENTS ON ACTIVITY OF THIOBACILLUS FERROOXIDANS

Most ores require a treatment by flotation before the ore concentrates can be used for leaching. Residues of mineral flotation reagents may remain in the ore and slurry; therefore, they may be carried over into the leaching process. Tuovinen (1978) and Dave and Natarajan (1982) investigated the effect of several flotation reagents on ferrous iron oxidation by Thiobacillus ferrooxidans. DOM (N-dodecyl mercaptan), KAX (potassium amylxanthate), KEX (potassium ethylxanthate), NABX (sodium butylxanthate), NAIPX (sodium isopropylxanthate), NAX (sodium amylxanthate), OULU 102 (pine oil), R-AMINAC C (Primary amine acetate), DOWFROTH 250 (polypropylene glycolmethyl ether), TEB (1,1,3-triethoxybutane), CMC(BDA) (carboxymethylcellulose), and DEXTRIN inhibited ferrous iron oxidation to varying degrees (Tuovinen, 1978). Tuovinen (1978) suggested that this wide variation of inhibition may indicate that the toxicity of the different reagents is dependent on their chemical composition and degradation in the acid environment.

Potassium ethyl xanthate, sodium isopropylxanthate, potassium amyl xanthate, pine oil and methyl isobutyl carbinol (MIBC) did not have any inhibitory effect on the ferrous iron oxidation rate at concentrations similar to those used in typical flotation practices (2×10^{-5} - 8×10^{-4} g/50ml) (Dave and Natarajan, 1982). At a concentration of 8×10^{-4} g/50ml, all of the reagents enhanced the iron oxidation by the microorganisms. Potassium ethyl xanthate enhanced the ferrous sulphate oxidation to the greatest degree.

The flotation reagents tested by Dave and Natarajan (1982) exhibited an inhibitory effect on zinc solubilization from an analar-grade zinc sulphide to varying degrees. There was not, however, any significant change in the rate of zinc extraction. Potassium amyl xanthate exhibited the greatest inhibitory effects by decreasing zinc solubilization by approximately 8% at a concentration of 5×10^{-4} g/50 ml. Pine oil had the least inhibitory effect. The researchers suggested that the inhibitory effects of the reagents on zinc solubilization could be explained by the adsorption of the chemicals on the solid-liquid interface. Potassium amyl xanthate was the most surface active among the flotation reagents used and therefore exhibited the greatest inhibition of bacterial activity.

EFFECT OF SURFACE ACTIVE AGENTS

The ability of Thiobacillus ferrooxidans to oxidize a chalcopyrite concentrate was reduced in the presence of the surface active agents Tween 20 (polyoxyethylene (20) sorbitan monolaurate), Tween 60 (polyoxyethylene (20) monostearate), and Tween 80 (polyoxyethylene (20) monooleate) (Torma et al., 1976). As the concentraton of the surfactants increased, the bacterial oxidation of the chalcopyrite, the specific rate of oxygen uptake, the oxygen concentration at saturation and the surface tension of the liquid medium decreased.

Torma et al. (1976) suggested that the inhibition of the chalcopyrite oxidation ability was a result of the decrease in the surface tension of the leach solution and the oxygen concentration at saturation.

EFFECT OF SOLVENT EXTRACTION ORGANICS

McCready (1977) tested the effect of four diluents, Solvesso 150 (95.3% aromatics), Escaid 100 (20% aromatics), Shell 140 (5% aromatics), Norpar (0.6% aromatics); an extractant, Alamine 336; and a modifier, isodecanol, on the activity of Thiobacilius ferrooxidans. These microorganisms were capable of growth and metabolism in the presence of 30% saturation of the solvent extraction organics at pH 2.0. Higher concentrations of the organics or deviations in pH from 2.0 resulted in growth inhibition or cellular lysis and death. The diluents and modifier were thought to inhibit growth by chemically attacking the cytoplasmic lipids of the cells. Alamine 336, an extractant reagent, complexed with ferrous iron, phosphate and ammonia and as a result, acted as a competitive inhibitor to cellular growth.

EFFECT OF A SOLVENT EXTRACTION FORMULATION ON COPPER RECOVERY RATES IN A THIOBACILLUS FERROOXIDANS SPK1 LEACHING SYSTEM

Babij and Madgwick (1983) found that a solvent extraction formulation (10% (v/v) of "Shell Metal Extractant 530" (R) in "Shellsol 2046" (R) hydrocarbon solvent) halved the copper recovery rates in the bacterial leaching of a chalcopyrite concentrate by Thiobacillus ferrooxidans SPK1. The researchers proposed that the removal of the solubilized copper from the aqueous to the organic phase caused inhibition of the rate of copper leaching due to its toxicity to the bacteria. They recommended that in commercial processes, the solvent should be added at the end of the batch leaching operation in order to eliminate its inhibitory effects.

WATER POTENTIAL AND THIOBACILLUS FERROOXIDANS

Brock (1975) suggested that water availability in leaching dumps may play an important part in controlling the growth and activity of *Thiobacillus ferrooxidans* and as a result in the effectiveness of the leaching operation. Brock (1975) investigated the effect of water potential on the growth of *Thiobacillus ferrooxidans* and compared the results with the water potentials found in pyritic substrates. The lower limit at which iron oxidation by the bacterium occurred was determined as -23 bars.

Water potentials of coal refuse materials varied widely due to the large variation in organic materials and clays that were present. Some coal refuse materials had water potentials at which *Thiobacillus ferrooxidans* could not grow. Brock (1975) suggested that the oxidizing activity of *Thiobacillus ferrooxidans* may promote the development of conditions in which moisture required for its own development will be limited. Clay mineral synthesis can occur by the breakdown of rock-forming minerals as a result of the acidic conditions produced from the oxidation of pyritic materials to sulphuric acid; therefore, the presence of clay minerals lowers the water potential.

In copper leach dumps, it is important that pore space is maintained in the pile since the bacterial oxidation of sulphide materials is an aerobic process. This may result in restricted water availability and inhibition of the growth of *Thiobacillus ferrooxidans*.

PHYSIOLOGICAL RESPONSE OF THIOBACILLUS FERROOXIDANS TO SIMULATED DEEP SOLUTION MINING CONDITIONS

Davidson et al. (1983) determined the effect of simulated deep solution mining conditions on the activity of *Thiobacillus ferrooxidans* . The maximum hydrostatic pressures that permitted the growth of *Thiobacillus ferrooxidans* was 30.4 MPa. Microbial growth was inhibited by hyperbaric oxygenation of the growth media at levels between 24 and 49 ppm O_2. Hydrostatic pressures of 0.10 to 68.9 MPa over a six hour period and helium pressures of 0.10 to 6.89 MPa did not affect the leaching of chalcopyrite by washed bacterial cell suspensions. The amount of copper released by growing cultures of *Thiobacillus ferrooxidans* decreased as hydrostatic pressure increased.

INFLUENCE OF PARTICLE SIZE ON ACTIVITY OF THIOBACILLUS FERROOXIDANS

The influence of ultra-fine particles (0.4 and .8 μm) obtained from the attributions grinding of a high-grade chalcopyrite concentrate on the activity of *Thiobacillus ferrooxidans* was examined by Torma and Rozgonyi (1980). The samples were leached by *Thiobacillus ferrooxidans* at pH 2.3 and 35°C for 7 days. At low pulp densities (less than 5%) 100% of the copper was extracted using the 0.4 and 0.8 particle size fractions. High extraction rates were also obtained with these small size fractions. The highest rate of copper extraction achieved in these experiments was 1,650 mg/l using the 0.4 μm size particles at 25% pulp density.

Torma and Rozgonyi (1980) suggest that almost complete copper extraction can be achieved from a high-grade, attrition ground substrate in low-pulp density solutions in one bacterial leaching step. Approximately 4.5 ml of the leach solution from the experiments was subjected to a solvent extraction procedure described by Torma et al. (1979). 99.5% pure copper was obtained by electroplating.

EFFECT OF METAL IONS ON THE ACTIVITY OF THIOBACILLUS FERROOXIDANS

Bacterial strains used in the leaching of minerals must be able to tolerate high concentrations of metal ions so that their activity is not adversely affected by the continuous increase of dissolved metal ion concentrations in a leaching operation.

The effect of different concentrations of metal ions characteristic of leaching solutions on the oxidative ability of Thiobacillus ferrooxidans was studied (Golomzik et al., 1974b). The daily average rate of iron oxidation was used to judge the effect of the ions. The metal ions were arranged according to their increasing inhibitory effect: zinc, sulphate ion, sodium, chlorine, magnesium, calcium, aluminum, silica and copper.

Nickel

At low concentrations, nickel did not affect growth, iron consumption rate, iron-oxidizing activity and sulphur-oxidizing activity (Golovko and Egorov, 1974; Imai, 1976). 2.0 g/l suppressed activity in the first few days of incubation (Golovko and Egorov, 1974).

Copper

Thiobacillus ferrooxidans was found to be more sensitive to copper ions than nickel ions (Golovko and Egorov, 1974). Copper ions (1.0 g/l) slightly affected the rate of bacterial oxidation of ferrous iron. The rate of ferrous iron oxidation was dramatically reduced with a copper concentration of 2.0-4.0 g/l (Golovko and

Egorov, 1974, Pol'kin et al., 1975). Copper ions at 8 g/l were toxic to the bacteria. The addition of small amounts of $ZnSo_4$ reduced the inhibitory effect of copper with respect to ferrous iron oxidation (Dave et al., 1979). Dave et al. (1979) suggest that the inhibition of iron oxidation may be the result of toxic metal ions binding at the same sites on the cell surface as ferrous ions. This decrease in copper toxicity on Thiobacillus ferrooxidans in the presence of zinc may be the result of the replacement of cupric ions in the sensitive sites of the cell walls by zinc ions. Thiobacillus ferrooxidans has been adapted to oxidize iron in the presence of < 160 g/l $CuSO_4$ (Dave et al., 1979). These results are contradictory to those of Imai (1976). Imai (1976) found that copper did not exhibit any inhibitory effects on the bacterium with respect to growth, iron consumption rate and iron-oxidizing activity.

Uranium

5 to 100 mM (1.2 to 2.4 g/l) uranium results in a loss of viability, a decrease in ferrous iron oxidation and an inhibition of carbon dioxide fixation by Thiobacillus ferrooxidans (Brierley, C.L., 1978b). This uranium toxicity is attributed to the loose-binding of uranium at iron-oxidation and metal-transport sites on the bacterial cell membrane. Uranyl sulphate ions did not inhibit iron-oxidizing activity at 10^{-4}, 10^{-3} and 10^{-2}M even though they almost completely inhibited growth at 5×10^{-3}M (Imai, 1976).

Tuovinen et al. (1980b) proposed that a plasmid may encode uranium resistance in Thiobacillus ferrooxidans. After examining the plasmid composition of eleven Thiobacillus ferrooxidans cultures which had been enriched from leach liquor samples, Tuovinen et al. (1980b) found that a plasmid of approximately 13.5 megadaltons was present in five of the cultures. With one uranium resistant strain, TFI-4, the 13.5 megadalton plasmid was detected after the strain had been enriched on ferrous sulphate. However, after subculturing, the plasmid was not detected and the strain became sensitive to 2mM uranyl sulphate. When the strain was re-adapted to grow in the presence of 2mM uranyl sulphate, the plasmid was once again detected.

Adaptation of Thiobacillus ferrooxidans to uranium

Guay and Silver (1980/81) isolated iron-oxidizing bacteria from a settling pond located at the base of a tailing dump. These bacteria were grown ferrous sulphate medium both in the absence and presence of 10 g/l uranium ore. The adapted and unadapted bacteria were then grown in 100 g/l of the uranium ore in shake flasks. At various times, the amount of dissolved uranium in the flasks was determined.

The results of this study showed that the adapted culture of the iron-oxidizing bacteria had a greater ability to solubilize uranium from a low-grade ore than its parent strain. Both the adapted and parent strains, however, had a greater ability to solubilize uranium than an authentic Thiobacillus ferrooxidans strain. After six days of leaching, the authentic strain had extracted less than 25% of the uranium while both the adapted and parent strains of the isolated bacteria extracted more than 75% of the uranium. Negligible amounts of uranium were solubilized in the sterile control. This indicated that no abiological uranium solubilization occurred.

Molybdenum

Duncan et al. (1967b) found that molybdenum was toxic to Thiobacillus ferrooxidans and only partial adaptation of this leaching bacterium to increasing concentrations of molybdenum could be achieved. Molybdenum oxide ions at 10^{-4} M and 10^{-3} M inhibited iron-oxidizing activity (Imai, 1976).

Mercury

Mercury is often associated with coal and metal ore deposits. Olsen et al. (1981) found that a strain of Thiobacillus ferrooxidans (designated BA-4) was very sensitive to mercury but successive subcultures at progressively higher mercury concentrations resulted in an increased resistance to mercury. The level of tolerance was increased from 0.05 µM (0.01 ppm) to greater than 10 µM (2.0 ppm). Further experiments found that the resistance involved the volatilization of mercury. The volatile mercury (Hg^0) was produced during the lag in the onset of iron oxidation in the presence of mercury. When the mercury concentration dropped to almost zero, measurable iron oxidation began. Olsen et al. (1981) suggested that this resistance to mercury could be plasmid mediated.

Silver

Soluble silver is toxic to Thiobacillus ferrooxidans (Hoffman and Hendrix, 1976). An adverse effect on growth was observed with a silver concentration of 0.1 ppm with complete inhibition in solutions containing 5.0 ppm silver. Inhibition of growth has also been observed at 10^{-7} M (Norris and Kelly, 1978a) and 10^{-3} M (Imai, 1976). This inhibition could be a problem when using this bacterium in the leaching of complex ores. Complex ores of copper, lead, zinc, etc, sometimes contain small amounts of silver in the form of the mineral argentite (silver sulphide). The presence of the silver would adversely affect the performance of this bacterium during bioleaching.

Arsenic

The minimum ionic concentrations of arsenic (III), arsenic (IV) at which the iron-oxidizing capacity of the bacteria was reduced, were, 1 g/l and 2 g/l, respectively (Pol'kin et al., 1975). By repeated subculturing, Pol'kin et al. (1975)

adapted a strain of Thiobacillus ferrooxidans to 2 g/l arsenic (III). Duncan et al. (1967b) suggested that arsenic is not likely to be toxic to the bacteria at concentrations found in commercial leaching operations.

Zinc

Dave et al. (1979) found that zinc concentrations above 40 g/l resulted in the inhibition of ferrous iron oxidation due to zinc toxicity. However, the bacteria were adapted and able to oxidize iron in the presence of 640 g/l $ZnSO_4$ (Dave et al., 1979). Imai (1976) found that at zinc concentrations of 10^{-3}M iron-oxidizing activity was not inhibited.

Cobalt and Chromium

Imai (1976) found that neither cobalt nor chromium at 10^{-3}M inhibited sulphur and iron oxidation.

Tin

Tin concentrations of 10^{-3} and 10^{-4}M inhibited iron oxidation but not sulphur oxidation (Imai, 1976).

Cadmium

10^{-3}M cadmium inhibited sulphur-oxidizing activity by 69% (Imai, 1976).

Lead

Lead nitrate at 10^{-3}M delayed growth and iron consumption for two days but did not inhibit iron- and sulphur-oxidizing activity (Imai, 1976).

DISTRIBUTION AND ACTIVITY OF NATURALLY-OCCURRING THIOBACILLUS FERROOXIDANS

Karavaiko et al. (1981) studied the distribution and activity of Thiobacillus ferrooxidans in solutions from an industrial plant for the dump leaching of copper at a copper-smelting complex. Low temperatures inhibited bacterial activity. The rate of ferrous iron oxidation was reduced two to five fold when the temperature was decreased from 20-25C to 8-12C. The maximum bacterial count was observed in the summer months (10^6-10^7/ml).

OCCURRENCE AND ACTIVITY OF THIOBACILLUS FERROOXIDANS IN A COMMERCIAL SCALE TEST PLOT FOR THE LEACHING OF COPPER AND ZINC

Golomzik et al. (1974a) studied the occurrence and activity of Thiobacillus ferrooxidans in a commercial-scale test plot for the underground leaching of copper and zinc. The ore body was found to have a deficiency in oxygen and mineral nutrients (phosphorus and ammoniacal nitrogen), a low temperature and an irregular, inadequate moisture content. The growth and activity of Thiobacillus ferrooxidans was extremely low under the above conditions. The leaching and oxidative abilities of the bacteria were optimized by developing an ore irrigation regime and by adding nitrogen and phosphorus salts.

INTRODUCTION OF A MUTANT STRAIN OF THIOBACILLUS FERROOXIDANS INTO A LEACHING DUMP

Maintaining suitable microflora in leached material is important in industrial leaching operations. Groudev et al. (1978) introduced a laboratory strain of Thiobacillus ferrooxidans (designated Pmv) possessing a high leaching activity into a small section of a copper ore dump in Bulgaria. Two years after the introduction of the strain, approximately 55% of all the Thiobacilli present were the Pmv strain. However, these ferrobacilli were only 4-5% of the total bacterial population in the dump and only a portion of the isolated Pmv-like bacteria retained the high leaching activity observed under laboratory conditions. As a result, the leaching kinetics in the experimental section of the dump did not increase in comparison to those of the control section.

MUTUALISM BETWEEN THIOBACILLUS FERROOXIDANS AND BEIJERINCKIA

Studies have confirmed the existence of a mutualistic relationship between Thiobacillus ferrooxidans and the nitrogen-fixing bacteria Beijerinckia lacticogenes (Trivedi and Tsuchiya, 1975; Tsuchiya et al., 1974). The Beijerinckia fixes dinitrogen to provide a nitrogen source for itself and Thiobacillus ferrooxidans. The Thiobacillus ferrooxidans fixes carbon dioxide to provide a carbon source for both organisms. The microbial mutualism has been observed to increase the rate and extent of microbial leaching of copper and nickel when compared to the rate and extent of leaching by Thiobacillus ferroxidans alone. A mixed culture of Thiobacillus ferrooxidans and Beijerinckia lacticogenes extracted over 70% of the copper and 100% of the nickel from a flotation concentrate over a 500 hour time period at pH 2.5 (Tsuchiya et al., 1974). The pure culture of Thiobacillus ferrooxidans extracted less than 25% copper and less than 80% nickel over the same time period and pH conditions.

Rangachari et al. (1978) also carried out leaching experiments using Thiobacillus ferrooxidans and Beijerinckia lacticogenes (NCIB 8846). Approximately 40-50% of the copper was extracted at the end of 40 days; however, the researchers did not feel that any significantly larger amount of copper had been extracted in the presence of Beijerinckia lacticogenes. This was contradictory to the findings of Tsuchiya et al. (1974).

IRON OXIDATION BY THIOBACILLUS FERROOXIDANS

DIRECT METHOD FOR THE CONTINUOUS DETERMINATION OF IRON OXIDATION

Steiner and Lazaroff (1974) developed a method for the direct, continuous determination of ferric ions produced during autotrophic iron oxidation which depends upon the measurement of ferric ion absorbance at 304 nm. The analysis is carried out as a continuous monitoring technique and does not require the reaction to be stopped or colour reagents to be added for analysis. A graphical and computer method were given for the determination of the ferric ion concentration at any time interval in reaction mixtures containing *Thiobacillus ferrooxidans* and ferrous ions at known SO_4^{2-} and hydrogen ion concentrations.

EFFECT OF NUTRIENTS ON FERROUS IRON OXIDATION

Rawlings (1981) found that the rate of ferrous iron oxidation in a continuous flow fixed-film system (used for the generation of ferrous iron in a uranium leaching plant) was not improved by the addition of nutrients (ammonium, potassium, phosphate).

EFFECT OF pH AND TEMPERATURE ON KINETICS OF Fe^{2+} OXIDATION

Moshnyakova and Karavaiko (1979) studied the effect of pH and temperature on the oxidation of ferrous iron by *Thiobacillus ferrooxidans.* At 28°C, both bacterial growth and ferrous iron oxidation occurred rapidly. The pH of the medium decreased during the active oxidation of ferrous iron as a result of the hydrolysis of ferric iron. At 12°C, the iron oxidation by the bacteria decreased 4 to 5 fold. The researchers concluded that the active oxidation of Fe^{2+} by bacteria depended on the conditions promoting the hydrolysis of Fe^{3+} and the liberation of H^+ ions.

Fe^{3+} was actively hydrolized at 28°C but weakly hydrolyzed at 12°C. As a result, at 12°C the pH increased and suppressed the activity of *Thiobacillus ferrooxidans*. Moshnyakova and Karavaiko (1979) felt that this indicated that the activity of *Thiobacillus ferrooxidans* at low temperatures was not determined by temperature but by pH.

INHIBITION OF IRON OXIDATION BY FINELY GROUND PARTICLES

Soljanto et al. (1980) found that finely ground particles (pyrite ore, uranium-bearing quartzite, fluorapatite, sea sand, pulverized quartz and glass beads) inhibited the iron oxidation activity of *Thiobacillus ferrooxidans* at pH values obtained in commercial leaching operations. The inhibition was related to the shaking of the culture flasks and to the type, amount and particle size of the various materials studied.

In contrast, Vuorinen et al. (1983) found that shaking, but not particle size or initial pH influenced the relative proportion of different iron species during the solubilization of iron during pyrite oxidation of *Thiobacillus ferrooxidans*.

Particle size of the pyrite influenced the solubilization of pyrite in *Thiobacillus ferrooxidans* cultures. The amount of iron solubilized increased as particle size decreased.

Tomizuka and Yagisawa (1978) observed a parallel relationship between an increase in the ferric iron concentration and bacterial cell numbers with *Thiobacillus ferrooxidans* growing in batch culture.

INHIBITION OF IRON OXIDATION ACTIVITY BY PYRITE

The inhibition of ferrous iron oxidation by pyrite may be important in systems involving bacterial leaching of ore. If the bacterial iron oxidation rates remained low in a leaching system, the metal leaching rates would also be inhibited.

Concentrations of granular pyrite particles (-50 mesh) greater than 1% (w/v) were increasingly inhibitory to ferrous iron oxidation by Thiobacillus ferrooxidans but this inhibition was removed by increasing the pH from pH 1.5 to pH 2.5 (Tuovinen et al., 1980a). High-grade pyrite was completely inhibitory to the ferrous oxidation at 1% concentration. An increase in pH only slightly removed the toxic effects. Pyrite toxicity was not alleviated by various concentrations of Tween 20, Tween 40 or Tween 80; the sulphates of NH_4^+, Na^+, K^+, Mg^{2+}, Cu^{2+} or 0.05% (w/v) yeast extract.

The sulphide ore concentrate and high-sulphur coal also inhibited ferrous iron oxidation. The sulphide ore exhibited more inhibition at pH 2.5 than the -50 mesh pyrite particles.

Washing the pyrite (-50 mesh) by soaking the particles in distilled water before exposure to the microorganisms, decreased the inhibitory effects of the pyrite on the oxidation process. The researchers concluded that some of the toxicity of pyrite was associated with a water-soluble component. Further studies, in which the pyrite particles were suspended in a mineral salts solution containing approximately 115 mM $FeSO_4$ and shaken for different lengths of time (1,8 and 24 hours) before filtration, indicated that the toxic component of the pyrite could be leached. The toxicity of the filtrates was related to the amount of pyrite soaked and the length of the soaking time. Autoclaving of pyrite or the filtrates for 15 min. at 120°C before innoculation with the Thiobacillus ferrooxidans culture also greatly reduced the inhibitory effects. The researchers suggested, that during autoclaving, the toxic component of the pyrite was oxidized or partly removed from the particle surface.

EFFECT OF THE CONCENTRATION OF FERROUS IRON ON ITS RATE OF OXIDATION

Kovrov et al. (1978) studied the effect of the concentration of ferrous iron on its rate of oxidation using *Thiobacillus ferrooxidans*. The specific oxidation rate of ferrous iron increased with an increase in its concentration in the culture medium and decreased with an increase in the total iron content. The cultivation of *Thiobacillus ferrooxidans* with the electrochemical reduction of ferrous iron produced high cell concentrations (4.5 g/l dry biomass weight) at a low concentration of total iron (4-6 g/l). The low total iron allowed for the high oxidation rates of the substrate and, as a result, high growth rate of the cells.

FERRIC IRON INHIBITION OF FERROUS IRON OXIDATION

Kelly and Jones (1978) found that the principle factor in limiting growth of a batch culture of *Thiobacillus ferrooxidans* on ferrous iron was inhibition by ferric iron product on the rate of ferrous iron oxidation.

Effect of CO_2 Availability on Growth of Batch Cultures

Exponential oxidation of $FeSO_4$ occurred in batch cultures in unsealed flasks. In sealed flasks containing normal air and labelled carbon dioxide ($14CO_2$), $FeSO_4$ oxidation was initially exponential but then became linear after 20-30% of the iron was consumed.

Inhibition of $FeSO_4$ oxidation by Fe^{3+}

Different concentrations of ferric sulphate were added to batch cultures of *Thiobacillus ferrooxidans* and ferrous sulphate. The ferric ions inhibited oxidation competetively. High concentrations of potassium partially alleviated the competitive inhibition by ferric ions.

Effect of high ferrous sulphate levels on Fe^{2+} oxidaton

As initial $FeSO_4$ concentration was increased from 62 to 249 mM, the lag period, before microbial growth occurred, increasd and rate of development decreased. This indicates that $FeSO_4$ could be inhibitory at high concentrations.

Iron oxidation by suspensions of *Thiobacillus ferrooxidans*

$FeSO_4$ oxidation increased up until about 100mM $FeSO_4$ but at higher concentrations, the rate decreased. This indicated $FeSO_4$ inhibition.

ANAEROBIC REDUCTION OF FERRIC IRON TO FERROUS IRON

Kino and Usami (1982) found that *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* were able to reduce ferric iron to ferrous iron when anaerobically grown on elemental sulphur as an energy source. Ferrous iron formed during the reduction by *Thiobacillus thiooxidans* may be converted to the ferric state by *Thiobacillus ferrooxidans* in an acidic environment.

MINERALS OXIDIZED BY THIOBACILLUS FERROXIDANS

Bruynesteyn and Duncan (1976) list the sulphide minerals that can be oxidized by *Thiobacillus ferrooxidans*.

Based on their own experience, Bruynesteyn and Duncan (1976) state that most nickel and zinc sulphides leach well. Chalcocite, covellite, bornite and tetrahedrite are the copper sulphides that leach the best. Chalcopyrite is unpredictable. Not all samples of pentlandite leach well. Galena and molybdenite do not leach to a degree considered to be of economical importance.

OPTIMUM LEACH GROWTH CONDITIONS FOR LEACHING BY THIOBACILLUS FERROOXIDANS (Torma and Banhegyi, 1984)

Parameters	Optimum Conditions
Temperature	28-55°C
pH	1.9-2.6
Eh	>500mV
(Fe^{2+})	3-8gldm^3
$(NH_4)_2SO_4$	3gldm^3
K_2HPO_4	0.5gldm^3
CO_2 in air	0.2%(v/v)
O_2	good aeration

Torma and Banhegyi (1984) suggest that the ore to be leached should be in fairly small particles to assure a fast release of the metal. When heap or dump leaching are practised, wet and dry periods should be alternated during the leaching process to remove larger particles and soluble salts from particle surfaces. The diffusion of oxygen and carbon dioxide to mineral surfaces will also be improved.

THIOBACILLUS THIOOXIDANS

Thiobacillus thiooxidans cells are short rods which may occur singly, in pairs or in short chains (Buchanan and Gibbons, 1974). As a strictly aerobic autotroph, it can carry out a rapid oxidation of elemental sulphur. Other partially reduced sulphur compounds can also be utilized (Buchanan and Gibbons, 1974). Ammonium salts serve as the nitrogen sources but the use of nitrates has been reported for two strains.

The temperatures for growth range from 10 to 37°C; the optimum temperatures for growth are 28 to 30°C. The optimum pH for growth is between 2.0 and 3.5; the upper limit of growth is near 6.0, the lower limit near pH 0.5.

Reference strain: K.R. Butlin 3/TA; NCIB 8343; ATCC 19377

Other names:	*Thiobacterium thiooxydans* (sic),
	Thiobacillus thermitanus
	Thiobacillus lobatus
	Thiobacillus crenatus
	Thiobacillus umbonatus
	Thiobacillus concretivorus

EFFECT OF CHROMIUM, MOLYBDENUM, VANADIUM ON *THIOBACILLUS THIOOXIDANS*

Vanadium (V), chromium (VI) and molybdenum (VI) affected the growth of *Thiobacillus thiooxidans* ATCC 8085 (Sullivan et al., 1980a). If these metals are released from mineral growth as a result of bacterial activity, the rate and extent of metal release may be decreased.

Both chromate (VI) and molybdenum (VI) suppressed growth at concentrations of 0.2 mM. Below these concentrations, a lag appeared in the growth curve, followed by normal growth. Vanadium (IV) did not adversely affect the growth of Thiobacillus thiooxidans. Vanadium (V) concentratons of 3-600 ppm caused a lag phase in the growth phase and concentration of 1,000 ppm completely inhibited growth. The vanadium (V) was reduced to vanadium (IV) during the bacterial growth as indicated by the change in colour (yellow to blue) in solutions of vanadium (V). The researchers suggested that the growth of the bacteria is suppressed until all of the vanadium (V) is reduced since the length of lag phase in bacteria growth almost coincided with the time required for reduction of vanadium (V). The vanadium (V) was only reduced in the presence of living cells.

OXIDATION OF SULPHIDE MINERALS BY THIOBACILLUS THIOOXIDANS

From studies conducted on the role of Thiobacillus thiooxidans in the oxidation of sulphide minerals, Karavaiko and Moshnyakova (1974) concluded that although different strains of pure Thiobacillus thiooxidans readily oxidize elemental sulphur, they are incapable of oxidizing sulphide minerals characteristic of copper-pyrite deposits (pyrite, chalcoyrite and ores containing chalosine, covellite, bornite and arensopyrite). The bacteria died or their concentration was greatly reduced.

THERMOPHILIC MICROORGANISMS

Some recent investigations have been directed towards the isolation and development of bacteria able to metabolize and to degrade minerals at temperatures in excess of those which optimize the growth of Thiobacillus ferrooxidans.

THIOBACILLUS TH1

Thiobacillus TH1 is an acidophilic, iron-oxidizing heterotroph. The optimum growth and oxidation of Fe^{2+} in a mineral salts medium requires an organic supplement of yeast extract (Brierley, J.A., et al., 1978). Growth was inhibited by 0.1% glucose in the presence of 0.02% yeast. Limited growth and Fe^{2+} oxidation occurs when the yeast extract is replaced by sodium thisulphate or potassium tetrathionate (Brierley, J.A., et al., 1978).

TH1 grows rapidly at 50°C on elemental sulphur, copper sulphide (CuS), pyrite, chalcopyrite, or a copper concentrate when the organic supplement is provided. The ferrous iron oxidation rate increases as temperatures increase above 50°C. The maximum rate for iron oxidation was obtained at 60°C (Brieirley, J.A., et al., 1978).

The presence of metals was found to affect both growth and iron oxidation. While TH1 tolerated greater than 1,000 ppm cadmium (Brierley, J.A. et al., 1978), uranium and copper concentrations of 4.2 mM and 15.7 mM, respectively, completely inhibited growth (Brierley, J.A., 1980). Normal growth occurred with 5 ppm molybdenum but there was no growth with 50 ppm molybdenum (Brierley, J.A. et al., 1978). Iron oxidation was inhibited by ferric irons at 0.015M and .15 M (Brierley, J.A. et al., 1978). Iron oxidation decreased by 25-30% at copper and nickel concentrations of 263 mM and 382 mM, respectively (Brierley, J.A., 1980). Uranium and molybdenum concentrations of 150mM and 5.2 mM, respectively, reduced iron oxidation by 33 and 84%, respectively (Brierley, J.A., 1980).

THIOBACILLUS TH2

Brierley and Lockwood (1977) conducted experiments which demonstrated the presence of thermophilic, iron-oxidizing bacteria in a large scale copper leach system. The source of the microorganisms was either the crushed mine waste and/or the leach solution from an active copper leach dump used in the experiments. These bacteria were designated as *Thiobacillus* TH2.

THIOBACILLUS TH3

Thermophilic, iron-oxidizing bacteria were isolated from samples of mine waste material collected at a copper leaching dump (Brierley, J.A., 1978). This Thiobacillus-like group of microbes has been designated as TH3. They are gram-negative, rod-shaped microorganisms that require an organic supplement such as yeast extract or cysteine for growth. Either ferrous iron or pyrite are used as a source of energy. During growth on pyrite, TH3 will decrease the pH of the medium indicating sulphide oxidation. The upper temperature limit for iron oxidation and growth is 55°C.

TH3 is approximately the same size as *Thiobacillus ferrooxidans*. Occasionally TH3 forms very long chains of cells.

EFFECT OF METAL IONS ON TH1, TH2, TH3

Thiobacillus-like microorganisms were isolated from surface samples from a copper leach dump in New Mexico (Brierley J.A., 1980). Approximately 2 Thiobacillus-like bacteria were found for every 2.4 x 10^7 Thiobacillus ferrooxidans per gram dry weight of dump material. These facultative thermophiles, identified as TH1, TH2 and TH3, were capable of oxidizing ferrous iron at 50°C, pH 1.6 in a leach solution supplemented with yeast extract.

Cellular respiration and iron oxidation were decreased in all three strains by copper and nickel concentrations of 363 mM and 382 mM, respectively. A zinc concentration of 382 mM had no effect on iron oxidation by TH1 but it decreased iron oxidation by TH2 and TH3. Uranyl ions at 150mM decreased iron oxidation by 33% for TH1. All three strains were sensitive to molybdenum. TH1 iron oxidation was affected the most as the oxidation process decreased by 84% at 5.2 mM molybdenum. TH2 and TH3 could still carry out iron oxidation at 10^4 mM molybdenum.

Growth studies on the TH strains in the presence of cupric and uranyl ions indicated that both of these ions could inhibit growth. TH1 and TH2 were inhibited by 15.2 mM copper but growth of TH3 occurred at 157.4 mM copper. Uranyl ion concentrations greater than 4.2 mM inhibited growth in all three strains.

PHYSIOLOGICAL RESPONSE OF TH3 TO SIMULATED DEEP SOLUTION MINING CONDITIONS

Davidson et al. (1983) determined the effect of simulated deep solution mining conditions on the activity of TH3. The maximum hydrostatic pressures that permitted the growth of TH3 was 25.3 MPa. Microbial growth was inhibited by hyperbaric oxygenation of the growth media at levels between 24 and 49 ppm O_2. Hydrostatic pressures of 0.10 to 68.9 MPa over a six hour period and helium pressures of 0.10 to 6.89 MPa did not affect the leaching of chalcopyrite by washed bacterial cell suspensions. Hyperbaric oxygen conditions decreased copper leaching by TH3.

THERMOPHILIC MICROORGANISM FROM A COPPER LEACH DUMP

Other thermophilic microorganisms have also been isolated. Murr and Berry (1979) isolated a natural, thermophilic microorganism from a copper sulphide leach dump. The microorganism was effective in catalyzing the release of copper in solution at 55°C and capable of oxidizing Fe^{2+} to Fe^{3+}. Observations by both scanning and transmission electron microscopy indicated that the microorganism differed in form and structure from Thiobacillus ferrooxidans and had a cell wall approximately half as thick and less complicated than that of Thiobacillus ferrooxidans. Two forms of the microorganism were observed: one rod shaped and the other spherical. A transitory, rod-sphere shape was also observed indicating pleomorphism.

Murr and Berry (1979) suggested that leaching in a large, field test, copper-bearing waste leaching facility progressed in the following manner: The waste copper body heated up due to ambient temperature and chemical reactions. After 150 days, the ambient temperatures and Thiobacillus ferrooxidans population reached a maximum and leaching reactions began. As oxygen consumption leveled off after 200 days, the rate of temperature increase declined and the Thiobacillus ferrooxidans population declined rapidly. The temperature inside the leaching column reached a maximum near the middle of the waste body (53 to 59°C). The majority of the oxygen consumed was in the upper cooler portions of the column where the Thiobacillus ferrooxidans population was high. Murr and Berry (1979) concluded that although the thermophile was present in the hotter areas of the column, it did not contribute significantly to the catalytic oxidation process since oxygen consumption was not as great in the hotter levels as it was in the cooler, upper levels.

LEACHING OF SULPHIDE ORES BY A THERMOPHILIC BACTERIUM

Le Roux and Wakerley (1980) examined the possibility of increasing metal extraction rates at elevated temperatures using a moderately thermophilic bacterial culture. The culture was isolated from an Icelandic hot spring (Le Roux, N.W., Wakerley, D.S. and Hunt, S.D. 1977, cited by Le Roux and Wakerley, 1980). These microorganisms were capable of degrading pyrite, pentlandite, violarite, chalcopyrite and sphalerite at 50°C.

SULFOLOBUS ACIDOCALDARIUS

Sulfolobus acidocaldarius bacteria appear as spherical, non-motile cells (Buchanan and Gibbons, 1974). As aerobic, facultative autotrophs, they can use elemental sulphur, yeast extract, glutamate and ribose as energy sources. While growth can occur from 55°C to 85°C, the optimum temperature range for growth is 70-75°C. The optimum pH for growth is 2-3.

Brierley (C.L., 1977) used two thermophilic bacteria, Sulfolobus acidocaldarius and "ferrolobus" to leach metals from porphyry copper samples. "Ferrolobus" was not classified but was similar to Sulfolobus acidocaldarius. Both microorganisms were able to oxidize reduced iron and sulphur compounds and grew on simple organic compounds and yeast extract. Leaching tests were performed in shake flasks at 60°C and within 60 days, "ferrolobus" extracted 51% of the copper from a

-65 mesh chalcopyrite concentrate containing 29% copper. Supplementing the "ferrolobus" with yeast extract and ferrous iron enhanced the copper extraction. Only 8% of the copper was extracted from the sterile control. Leaching tests were conducted in glass leaching columns at 60°C on a chalcopyrite ore containing copper, zinc, lead, molybdenum and iron. Mixed cultures of Sulfolobus acidocaldarius and "ferrolobus" extracted 38% of the copper from the -3+100 mesh ore. The average rate of extraction was 21 mg/l/day in comparison to 1.9 mg copper/l/day for 4% extraction in the sterile control.

KINETICS OF LEACHING CHALCOPYRITE-BEARING WASTE ROCK WITH THERMOPHILIC AND MESOPHILIC BACTERIA

Chakraborti and Murr (1980) studied and compared the leaching characteristics of the thermophiles Sulfolobus acidocaldarius and "ferrobolus" with that of the mesophile Thiobacillus ferrooxidans. The leaching experiments were conducted in laboratory scale shake flask leaching systems on -4 to +6 mesh chalcopyrite waste rock. The optimum leaching temperature for Thiobacillus ferrooxidans was between 30 and 40°C; the optimum temperature for thermophile leaching was between 50 and 60°C. The thermophilic microorganisms were observed to function as take-over microorganisms with increasing temperature. Precipitates formed in the flasks after leaching for 6 weeks and consisted of gypsum microcrystals intermixed with agglomerates of bacteria, elemental sulphur and iron precipitates, jarosites and other mineral particulates with some attached bacteria (particularly thermophiles).

The activation energy for the thermophiles was 22.22 kcal/mol as compared to 26.77 kcal/mol for the mesophile Thiobacillus ferrooxidans. Chakraborti and Murr (1980) concluded that this difference in activation energy, together with the difference in optimum temperature, contributed to the higher reaction rate achieved by the thermophiles. The reaction order for Thiobacillus ferrooxidans was 0.92 compared with 1.29 for the thermophiles. The researchers felt that this may suggest that the mechanisms of reaction may differ. The rate of reaction increased by 1.93 for the thermophiles and 1.83 for Thiobacillus ferrooxidans for each 10°C temperature increase, indicating an enhanced catalysis by the thermophiles over Thiobacillus ferrooxidans.

COMPARISON OF THE LEACHING ABILITIES OF THIOBACILLUS FERROOXIDANS AND A SULFOLOBUS-LIKE THERMOPHILE

The leaching abilities of Thiobacillus ferrooxidans at ambient temperatures and a Sulfolobus-like microorganism at 60°C were compared in bench-scale copper leaching experiments (Brierley and Brierley, 1978).

Generally, the thermophilic microorganism extracted copper from the waste material, ores and concentrates tested better than Thiobacillus ferrooxidans. The differences in the leaching rates between the two microorganisms was attributed to leaching temperature differences and not to a greater catalytic activity by the Sulfolobus-like bacteria (Brierley and Brierley, 1978; Berry and Murr, 1978). However, the thermophilic Sulfolobus-like microbe did demonstrate a greater ability for extracting copper from a mineral rich in chalcopyrite (Brierley and Brierley, 1978). After 60 days, the thermophile had extracted 78% of the copper at 60°C from a chalcopyrite containing 29% copper as compared to 8.4% by Thiobacillus ferrooxidans at room temperature.

SULFOBACILLUS THERMOSULFIDOOXIDANS

An aerobic, facultatively thermophilic bacterium similar to Thiobacillus ferrooxidans was isolated from a spontaneously heated ore of a copper-zinc-pyrite deposit in the USSR (Golovacheva and Karavaiko, 1978). The differences of this bacterium with respect to Thiobacillus ferrooxidans led to the microorganism as being identified as a new species of a new genus, Sulfobacillus thermosulfidooxidans gen. nov., sp. nov. Golovacheva and Karavaiko (1978) suggest that, given the thermophilic nature of the bacterium, Sulfobacillus thermosulfidooxidans may play an active role in the oxidation of sulphide ores and in the warming of the ores in the deposits.

Sulfobacillus thermosulfidooxidans cells are gram-positive, non-motile rods which occur singly, as pairs, or in short chains. The spores are spherical or slightly oval. It is a strictly aerobic bacterium and does not grow anaerobically in the presence of nitrates. As a facultative thermophile, the bacterium is active in the temperature range 28-55°C. As a facultative autotroph, the microorganism can actively oxidize elemental sulphur, iron and sulfidic minerals (pyrite, arsenophyrite, chalcopyrite, sphalerite, antimonite, covellite, galenite) in the presence of yeast extract. This bacterium can grow heterotrophically on 0.1% glucose or sucrose but often an adaption period is required. The activity of the microorganisms in oxidizing sulphide ores is increased in the presence of glucose and glutamate.

LEACHING OF MINERALS USING BACTERIA OTHER THAN THIOBACILLI

Le Roux et al. (1978) investigated the feasibility of leaching copper-nickel sulphide concentrates using bacteria other than the commonly used thiobacilli. The mobile rod-shaped bacteria of different sizes were isolated from soil samples but were not examined in detail. Although the bacteria were similar in appearance to Thiobacillus ferrooxidans, no growth was obtained when the bacteria were grown on ferrous iron or mixtures of ferrous iron and malt and peptone media.

The amount of nickel and copper extracted was between 81-98% and 25-42%, respectively. Maximum nickel extraction occurred at pH 5.0 in an aerated flask. Efficiency of nickel extraction decreased with an increase in pH and pulp density.

The rate of nickel solubilization by the unknown bacteria was compared to that of Thiobacillus ferrooxidans. Thiobacillus ferrooxidans leached approximately 95% of the nickel in 50 days. The unknown bacteria released 98% of the nickel in 66 days. The researchers attributed this difference in leaching rates to the fact that Thiobacillus ferrooxidans has been adapted to high concentrations of metals over long periods of time. The soil isolates did reduce their leaching time by a factor of 3.5 with subculturing and the researchers feel that further adaptation would increase their activity and tolerance to metals.

ANAEROBIC LEACHING OF COPPER WITH LACTOBACILLI

Fresh whey was tested as a nutrient medium and as a source of lactic acid producing microorganisms (lactobacilli) for the anaerobic leaching of copper (Kiel and Schwartz, 1980). Up to 83.4% (600 ppm) of the copper was extracted from a suspension containing 5 g of copper ore in 60 ml of whey after 4 weeks. When the pulp density was increased to 50 g of ore/40 ml whey, only 18.3% of the total copper was solubilized after 4 weeks incubation time. A greater quantity of copper, however, was solubilized from the higher pulp density solution. After longer incubation periods, the amount of soluble copper decreased as the result of its precipitation or readsorption to the ore and gangue material.

LEACHING WITH DENITRIFYING BACTERIA

Copper silicate (e.g. chrysocolla) and carbonate-containing ores (malachite and azurite) are not entirely amenable to acid leaching due to the high consumption of acid and the formation of undesirable by-products which are difficult to treat; however, these ores can be leached using an aqueous nutrient solution containing a water soluble inorganic nitrate and denitrifying microorganisms (Zajic, 1966). Other metals such as cobalt, nickel, lead, cadmium and zinc may also be recovered by this invention. The denitrifying microorganisms are capable of reducing the nitrate ion to ammonia to extract the metal values and produce an aqueous medium containing dissolved metal values. Both heterotrophic and autotrophic denitrifying bacteria may be used. The heterotrophic bacteria include Bacillus denitrificans, Micrococcus denitrificans and Pseudomonas denitrificans. Hydrogenomas facilis and Thiobacillus denitrificans are examples of autotrophic bacteria which may be used. For best extraction results, the temperature and pH of the medium should be maintained at 25-30°C and 8, respectively, as the denitrifying microorganisms multiply most rapidly under these conditions.

An experiment was conducted to demonstrate the ability of denitrifying microbes to increase the amount of copper leached from an ore (Zajic, 1966). Two inoculated flasks containing 16.0 grams copper ore per 100 millilitres of leaching medium yielded 111 mg/litre of solubilized copper. On the other hand, only 35 mg/litre of solubilized copper was present in the sterile control flask.

RECOVERY OF METALS FROM A BACTERIAL LEACHING SOLUTION WITH SULPHATE-REDUCING BACTERIA

Tomizuka and Yagisawa (1973) studied the recovery of metals from acid mine drainage and bacterial leaching solutions that were characterized by high sulphate, metal and hydrogen ion concentrations. A mixed culture of sulphate-reducing bacteria was used in the experiments. A metal recovery system was developed using a continuous culture.

The rate of removal of the metals (Fe, Cu and Zn) from bacterial leaching solutions was greatly affected by pH. The optimum pH was 6.0 which was also the optimum pH for specific growth. The maximum rate of metal removal was 47.5 mg/l/hr for copper, 18.1 mg/l/hr for zinc and 25.7 mg/l/hr for iron.

A black precipitate containing 19.96% copper, 6.13% zinc and 10.95% iron was formed. The ratios of these metals in the precipitate were similar to those in the original leaching solution.

BACTERIUM CATALYZES IRON OXIDATION AT pH 4.5-3.5

A filamentous iron bacterium of the genus Metallogenium catalyzed iron oxidation in the pH range of 4.5 to 3.5 (Walsh and Mitchell, 1972). An examination of the ecological role of this bacterium showed it to be a member of a pH-dependent succession of iron bacterium. Growth of Thiobacillus ferrooxidans in acid mine waters was found to be dependent on the growth of Metallogenium to lower the pH of the solution through the acidity production from the ferric iron reaction following ferrous iron oxidation.

LEACHING WITH FUNGI

SOLUBILIZATION OF METAL OXIDES BY ASPERGILLUS NIGER

Strains of the acid-producing fungi Aspergillus niger solubilized numerous metal oxides (Kiel and Schwartz, 1980). Yttrium (III) oxide was solubilized the most under the experimental conditions, followed by canthanum (III)-, dysprosium (III)-, samarium (III)-, and neudymium (III) oxide.

LEACHING COPPER WITH FUNGI

Fungi involved in the solubilization of copper do not require iron or sulphur as an energy source but they do require an organic nutrient. The fungal processes operate in neutral to slightly acidic conditions and produce metabolites which solubilize copper by chelation (Wenberg et al., 1971).

LEACHING COPPER WITH ASPERGILLUS NIGER ATCC 9142

Surface cultures of Aspergillus niger ATCC 9142 leached 87.3% of the total copper from a 10% copper ore suspension within 2 weeks (Kiel and Schwartz, 1980). The main ore minerals were chrysocoll and malachite. The metal solubilization was attributed mainly to citric acid, a fairly strong metal complexing agent. Aspergillus niger produces citric acid as well as other organic acids such as oxalic, gluconic and itaconic acid.

Surface cultures were more effective in leaching copper from the ore than submerged cultures. In submerged cultures, growth of the fungal mycelia was suppressed by the rotation of the ore in shaking cultures. As a result, only 54.25% of the copper was solubilized by the submerged culture compared to the 87.3% extraction obtained with a surface culture.

LEACHING OF COPPER AND ZINC

Aspergillus niger and Paecilomyces varioti were the most active fungi involved in the dissolution of copper and zinc from their oxidized ores (Dave et al., 1981a). The leaching of the copper from oxidized ores was attributed to the production of citric and oxalic acids which act as chelating agents for the copper. Aspergillus niger was not active on sulphide copper ores (e.g. chalcopyrite) under the experimental conditions. The ability to extract zinc was not as significant as for the copper.

Metal solubilization was dependent on the ability of the fungi to adapt to a particular form of a sugar and the resulting synthesis of the metabolic end products. The role of a carbon source (sucrose or molasses) in copper and zinc dissolution by Aspergilus niger was demonstrated in leaching studies performed on an oxidized copper ore and an oxidized lead-zinc ore. Sucrose was found to be the better carbon source for metal solubilization. With the lead-zinc ore, 27% of the zinc was extracted with sucrose as the carbon source as compared to 14.7% zinc extracted with molasses as the carbon source. 31.9% of the copper was extracted with the sucrose carbon source; 4.2% copper was extracted with the molasses carbon source.

Metal extraction decreased with an increase in pulp density during experiments on the leaching of oxidized copper ore by Aspergillus niger. With a 2% pulp density, 65% of the copper was extracted. Only 9.3% of the copper was extracted when the pulp density was increased to 8%.

The overall effect of pH on copper leaching by Aspergillus niger was compared to chemical acid leaching. The results indicated that acid leaching alone made a major contribution to the copper solubilization.

METAL TOXICITY

The tolerance of acidophiles to most metals in low pH media results from the effective competition by H^+ ions for negatively-charged sites at the cell surface (Norris and Kelly, 1978a).

Tolerance of an organism to a metal is determined by comparing the oxygen uptake of the organism when it is suspended in varying concentrations of the soluble metal to the oxygen uptake when no metal is present.

SILVER TOXICITY

Metal toxicity could affect the activity of bacteria involved in mineral leaching. Norris and Kelly (1978a) studied silver toxicity for Thiobacillus ferrooxidans, Thiobacillus thiooxidans, Leptospirillum ferrooxidans and a thermophilic iron- and sulphur-oxidizing Thiobacillus-like bacterium isolated from Icelandic thermal springs. Silver nitrate ($AgNO_3$) concentration of 10^{-9}M slightly inhibited the growth of Thiobacillus ferrooxidans at pH 1.5. More severe inhibition was observed with increasing $AgNO_3$ concentration with growth completely inhibited after an addition of 10^{-7}M $AgNO_3$. The toxicity was alleviated by increasing the pH but not by adding potassium or sodium to the medium. The thermophilic bacterium was less sensitive to silver than Thiobacillus ferrooxidans but this was attributed to the components of yeast extract in the growth medium which complexed with the silver and reduced its soluble concentration. Leptospirillum ferrooxidans was the least sensitive to silver but some inhibition for all the organisms was observed by 10^{-6}M.

The high toxicity of silver to Thiobacillus ferrooxidans oxidizing Fe^{2+} was related to the accumulation of the metal by the cells. The extent of silver accumulation and rate of inhibition of Fe^{2+} oxidation was proportional to the silver concentration. Silver accumulation also affected the levels of major cell cations, particularily magnesium and potassium.

TOLERANCE OF A YEAST TO MERCURY

A yeast of the genus Cryptococcus was able to grow in a broth supplemented with high concentrations of mercury (II) chloride (180 mg of mecury per litre) (Brunker and Bott, 1974). The cells were able to metabolize glucose in the presence of mercury as did cells grown in the absence of mercury. The mercury was associated with the cell wall and membrane, and in vacuoles within the cytoplasm. The researchers suggested that the presence of high concentrations of mercury in viable cells indicated that the yeast had the ability to convert the mercury to its nontoxic form, elemental mercury.

TOLERANCE LEVEL OF BACTERIA

Tolerance levels of bacteria for specific ions can be increased by mutation. The tolerance level can be increased by growing a culture in a solution containing the metal ion at concentration first below the tolerance level. The individuals with high tolerance levels will survive and multiply to produce a culture with an increased tolerance level. This process can be repeated several times in solutions with increasing metal levels until a culture is produced with a high tolerance to a metal.

Strains of bacteria have been developed to tolerate 6,290 ppm aluminum, 4,975 ppm calcium, 2,400 ppm magnesium, 3,280 ppm manganese and 160 ppm molybdenum (Zimmerley et al., 1958).

ATTACHMENT OF MICROORGANISMS TO MINERAL SURFACES

Although there is considerable controversy as to whether or not a direct reaction occurs at a mineral surface, there is an advantage to the organism being attached to the surface since it would be proximal to the oxidizable products of leaching (Fe^+ and S) (Kelly et al., 1979). Several researchers have investigated the attachment of bacteria to mineral surfaces.

Berry and Murr (1978) studied the mode and characteristics of the attachment of bacteria to mineral surfaces using transmission and scanning electron microscopy. A Sulfolobus-like thermophile attached much more readily to pyrite and chalcopyrite surfaces than either Thiobacillus ferrooxidans or Thiobacillus thiooxidans. This was attributed to the greater mobility of the Thiobacilli due to their flagellum and to the fact that the cell wall of Sulfolobus-like microorganisms are less rigid and more conducive to attachment. Berry and Murr (1978) found some evidence that the Thiobacilli secreted a substance that may aid in their attachment. The nature of the substance is not known.

Bennett and Tributsch (1978) observed pyrite crystals that were exposed to the action of Thiobacillus ferrooxidans for 2 years with scanning electron microscopy. These studies revealed bacterial corrosion pits in characteristic patterns. Bennett and Tributsch (1978) suggested that the pit formation on the surface of a metal sulphide in contact with bacterial cells was a result of the activity of a bacterially-generated, chemical agent that increased the dissolution rate of the metal sulphide in localized areas. This bacterial agent could act in three ways: by oxidation of Fe^{2+} to Fe^{3+}, by solubilizing molecular sulphur on the surfaces or by a direct attack on the crystallized metal sulphide surfaces. The researchers felt that it was most likely that the pits were formed by the bacterial breakdown of the sulphur layer that forms after sulphur particles are placed in an acidified solution containing O_2 and that can limit the chemical dissolution of metal sulphides.

Myerson and Kline (1983) studied the adsorption of Thiobacillus ferrooxidans (ATCC 1361) on coal particles. Up to 90% of the bacteria were adsorbed during the first five minutes of exposure. At low bacterial concentrations, the bacteria were completely adsorbed onto the coal surface. This adsorption was irreversible. Myerson and Kline (1983) suggested that the irreversibility of the adsorption of Thiobacillus ferrooxidans on coal particles and the rapid rate at which the adsorption occurs were important parameters to be employed in the modeling of bacterial coal desulphurization processes.

It has not been established as to whether or not the attachment site is the result of specific selection of regions of specific ion concentration, changed stoichiometry or strained regions containing crystal defects such as dislocations, grain boundaries, cracks, etc., or the result of random selection.

Bennett and Tributsch (1978) found that the substrate provided parameters that affected bacterial organization under certain circumstances. The bacteria appeared to have the ability to distinguish between surface areas that mechanically or chemically deviated from ideally crystallized sulphide substrates. It may be to the advantage of the bacteria to obtain energy from mineral surfaces that are characterized by either weak chemical bonding or by an increased surface area for chemical interaction along cracks or polycrystalline regions. Based on these theories, Bennett and Tributsch (1978) concluded that Thiobacillus ferrooxidans was able to distinguish between favourable and less favourable sites on a mineral surface for energy extraction and selected the site of attack according to the availability of the nutrient. These conclusions have been supported by other researchers. Using transmission electron and scanning electron microscopes, Murr and Berry (1976) observed the systematic adsorption of bacteria to $CuFeS_2$ phase regions in a low-grade chalcopyrite ore. In a study on the bacterial leaching of Duluth Gabbro copper-nickel sulphides, Thiobacillus ferrooxidans attached themselves onto all sulphide mineral substrates (Natarajan and Iwasaki, 1983a). When Sulfolobus-like microorganisms, Thiobacillus thiooxidans or Thiobacillus ferrooxidans attached to the surface of a polished section of a low-grade waste rock, the attachment was specific to FeS_2 and $CuFeS_2$ surface regions (Berry and Murr, 1978). The attachment of the Thiobacilli was not as frequent and the density of the attached microbes was less than for the Sulfolobus-like microorganisms; however, the attachment did not seem necessary for enhanced solubilization of copper.

In contrast, Berry and Murr (1978) found that it was difficult to associate the attachment site of Sulfolobus-like microorganisms with any crystal defects, particularly dislocations. Although Wakao et al. (1984) found that Thiobacillus ferrooxidans cells eroded pyrite ore surfaces in characteristic patterns similar to those described by Bennett and Tributsch (1978) and Hiltunen et al. (1981), there was no prominent attachment of the cells to eroded surfaces and the surfaces appeared to be very heterogenous with respect to their affinity for bacteria. Wakao et al. (1984) also observed that bacterial iron oxidation was depressed by the attachment of Thiobacillus ferrooxidans to solid surfaces and proposed that the bacterial concentration in the aqueous phase rather than on the pyrite particles played a major role in the enhanced oxidation of pyrite in the presence of Thiobacillus ferrooxidans.

SECTION I.2

GALVANIC INTERACTIONS

GALVANIC INTERACTIONS

When two sulphide minerals of different rest potential are in contact with each other in an acidic solution, the mineral with the higher rest potential acts as a cathode and is galvanically protected. The mineral lower in the electromotive series dissolves rapidly (Mehta and Murr, 1983).

GALVANIC REACTIONS (Mehta and Murr, 1983)

$$\text{cathode reaction } \tfrac{1}{2}O_2 + 2H^+ + 2e^- = H_2O$$

The mineral with the lower rest potential acts as an anode.

$$\text{anode reaction } MS = M^{2+} + S^o + 2e^-$$

MS = metal sulphide

M = bivalent metal

Mehta and Murr (1983) found that in the leaching of concentrate pulp mixtures of sulphides having different rest potentials, galvanic interactions contribute significantly to the leaching process. This effect was enhanced in the presence of bacteria which accelerated the reaction by catalyzing oxidation reactions.

Berry et al. (1978) suggested that "bacteria do not preferentially attack sulphide minerals, but rather bacteria significantly enhance the preferential leaching of sulphides undergoing anodic reactions as a result of galvanic contact with pyrite. The presence of bacteria accelerates the galvanic conversion of the lower-rest-potential sulphides in pyrite-coupled sulphide systems by converting Fe^{2+} to Fe^{3+}

(to promote lixiviant activity) and by solubilizing elemental sulphur (thereby preventing a reduction in surface reaction by the formation of a sulphur layer)".

Galvanic conversions would have a greater significance in a low-grade waste heap or dump leaching operation because if a waste rock regime contained a large portion of contacting sulphides, the leaching rate would be largest for the sulphide member having the lower rest potential (Berry and Murr, 1978).

GALVANIC INTERACTIONS BETWEEN CHALCOPYRITE AND PYRITE

Several researchers have studied the galvanic interactions between minerals. Berry et al. (1978) studied the galvanic interaction between chalcopyrite and pyrite in a low-grade, waste-rock matrix during bacterial leaching in a lixiviant containing thermophilic, Sulfolobus-like microorganisms at 60°C and a lixiviant containing Thiobacillus ferrooxidans at 28°C. There was a major difference in bacterial leaching using the thermophilic microbes at 60°C but Berry et al. (1978) concluded that this difference was not due to differences in the intrinsic catalytic ability of efficiency of the microorganism but a result of enhanced kinetics at a high temperature. The leaching of chalcopyrite was enhanced in contact with pyrite because the resulting galvanic interaction caused the chalcopyrite to corrode more rapidly than the pyrite. This effect was accelerated as a result of the oxidation of sulphur by bacteria to sulphuric acid.

When pyrite and chalcopyrite phases are in contact, O_2 is reduced on the cathodic pyrite surface.

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \text{ (Pyrite only acts as a site for this reaction)}$$

On chalcopyrite surface, there is anodic reaction:

$$CuFeS_2 \rightarrow Cu^{2+} + Fe^{2+} + 2S^o + 4e^- \text{ (2S° oxidized by bacteria)}$$

GALVANIC DISSOLUTION IN SULPHIDE MINERAL COMBINATIONS

Natarajan and Iwasaki (1983b) found that the rest potentials of chalcopyrite-cubanite, pentlandite and pyrrhotite minerals at a pH of 2.5 were +250 mV, +180 mV to +100mV, and +120 mV to +110 mV, respectively. This indicated that in a chalcopyrite-cubanite-pentlandite-pyrrhotite combination, preferential oxidation of pentlandite and pyrrhotite would be expected.

Preferential dissolution of nickel over copper from a Duluth gabbro copper-nickel sulphide in the presence of Thiobacillus ferrooxidans was observed. This was a result of the preferential galvanic dissolution of pyrrhotite and pentlandite in contact with chalcopyrite-cubanite and the presence of the bacteria that catalyze the dissolution reaction. Natarjan and Iwasaki (1983b) suggested that bulk copper-nickel concentrates may be selectively leached for nickel by bacteria as a result of galvanic interactions. They found that nickel-adapted strains extracted approximately 90% of the nickel from a sample of Duluth gabbro within 45 days. Nickel release took place before copper appeared in the leaching solution.

MODEL OF COMBINED BACTERIAL-GALVANIC OXIDATION IN A CHALCOPYRITE-PYRRHOTITE CONTACT (Natarajan and Iwasaki, 1983b)

$FeS = Fe^{++} + S^o + 2e$ (anodic dissolution of pyrrhotite)

$O_2 + 4H^+ + 4e = 2H_2O$ (cathodic reduction of oxygen at chalcopyrite surface)

$(Fe_xNi\ 1-x)_9S_8 = 9xFe^{++} + 9(1-x)Ni^{++} + 8S^o + 18e$ (anodic dissolution of pentlandite in contact with chalcopyrite-cubanite)

Fe^{++}, S and S^0 are energy substrates for Thiobacillus ferrooxidans. The elemental sulphur formed on mineral surfaces due to oxidation will be oxidized by the bacteria to produce an acidic ferric sulphate lixiviant and to expose fresh mineral surfaces.

GALVANIC INTERACTIONS AND CHALCOCITE

Yakhontova and Nesterovich (1978) studied the bacterial oxidation of chalcocite. Divalent copper was extracted into solution and its extraction was accompanied by a constant increase in the pH of the leaching solution.

Yakhontova and Nesterovich (1978) suggested that the reaction of a bacterial cell with a sulphide particle follows the laws of an electrochemical model. The sulphide undergoes anodic conversion and the membrane systems of the cell play the part of the cathode, gathering the oxidation-reaction electrons.

DISSOLUTION OF COPPER AND ZINC SULPHIDES

When a leaching system contained a mixture of copper and zinc sulphides, Dave et al. (1979) found that electrochemical mechanisms came into play in selective metal dissolution. Galvanic effects in the multi-metal systems were considered. The rest potentials of the zinc sulphide and copper sulphide in the system were +140 mV and +340 mV, respectively. This indicated that the zinc sulphide was less noble than the copper sulphide and therefore, an enhanced dissolution of zinc in the presence of copper sulphide was expected. Experiments with leaching systems containing 5% zinc sulphide and various concentrations of copper sulphide confirmed this theory. The rate of zinc dissolution increased in the presence of copper sulphide in both control and innoculated systems. The rate of dissolution was dependent upon the relative amounts of the constituents in the leaching system. For example, in the presence of equal portions of zinc and copper sulphides, no copper dissolution was observed during a 26 day study. This indicates that selective dissolution of zinc or copper could be achieved through bacterial oxidation by proper use of adapted strains.

ROLE OF IRON IN METAL SULPHIDE LEACHING BY GALVANIC INTERACTION

Murr and Mehta (1983) concluded that galvanic conversion was a significant leaching phenomenon that was not influenced by iron concentration for specific system components. While ferric iron (as generated by bacterial activity) can play a role in the leaching of copper from $CuFeS_2$, it does not account for the enhanced leaching that occurs when $CuFeS_2$ is in contact with FeS_2.

There is no ferric sulphate production in ZnS leaching (Murr and Mehta, 1983). Total iron in solution after 28 days of sterile and bacterial leaching of ZnS/FeS_2 did not rise above 1% of the available iron. The leaching of ZnS/FeS_2 was attributed solely to galvanic conversion. The bacteria only accelerated the rate of reaction by adding the catalytic oxidation of ZnS to the galvanic conversion of ZnS.

SECTION I.3

PYRITE OXIDATION

BACTERIAL OXIDATION OF PYRITE

Yakhontova et al. (1980) state that the mechanisms of bacterial interaction with minerals is one of the most difficult and inadequately studied problems in geochemistry.

The oxidation of pyrite by Thiobacillus ferrooxidans has been described as an electrochemical one (Yakhontova et al., 1980). The sulphide is acted upon by reactions catalyzed by bacteria. These reactions supply the bacterial cells with electrons to take part in their "cathode" reactions. As a result, it is possible to describe the bacterial oxidation of the sulphide in terms of the mineral's electrode potential (EP) and the oxidation potential (OP) and pH of the culture solution.

PYRITE OXIDATION BY THIOBACILLUS FERROOXIDANS (Fenchel and Blackburn, 1979).

The first step in pyrite oxidation by Thiobacillus ferrooxidans is the oxidation of S^{2-}:

$$(1)\ 2FeS_2(s) + 2H_2O + 7O_2 \longrightarrow 2Fe^{2+} + 4SO_4^{\ 2-} + 4H^+$$

The ferrous iron is then oxidized by the bacterium by the following reaction:

$$(2)\ 4Fe^{2+} + O_2\ 4H^+ \longrightarrow 4Fe^{3+} + 2H_2O$$

The ferric iron formed will remain in solution in acidic environments. It is also a strong oxidant itself and will abiologically oxidize pyrite:

$$(3)\ FeS_2 + 14Fe^{3+} + 8H_2O \longrightarrow 15Fe^{2+} + 2SO_4^{\ 2-} + 16H^+$$

The ferrous iron which is produced serves as a subtrate for further oxidation by Thiobacillus ferrooxidans by reaction (2). As the pH of the solution increases, ferric iron will become hydrated and precipitate as hydrous ferric oxide:

$$(4)\ Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3(S) + 3H^+$$

This entire pyrite oxidation process can occur abiologically but the rate is increased by the activity of thiobacilli. The bacteria accelerate the oxidation of ferrous iron which is normally the rate-limiting step in the oxidation process. Examples of solubilization of metal ores by ferric iron (Fe^{3+}) (Fenchel and Blackburn, 1979).

Chalcopyrite

$CuFeS_2(s) + 4Fe^{3+} + O_2\ 2H_2O \rightarrow Cu^{2+} + 5Fe^{2+} + 4H^+ + 2SO_4{}^{2-}$

Sphalerite

$2ZnS(s) + 4Fe^{3+} + O_2 + 2H_2O \rightarrow 2An^{2+} + 4Fe^{2+} + 4H^+ + 2SO_4{}^{2-}$

Galena

$2PbS(s) + 4Fe^{3+} + O_2 + 2H_2O \rightarrow 2Pb^{2+} + 4Fe^{2+} + 4H^+ + 2SO_4{}^{2-}$

Uranite

$UO_2(s) + 2Fe^{3+} \rightarrow UO_2{}^{2+} + 2Fe^{2+}$

MECHANISM OF BACTERIAL PYRITE OXIDATION

Silverman (1967) suggested that both indirect and direct contact mechanisms operated in the oxidation of pyrite. Support for the indirect contact mechanism was given by the experimental observation that the removal of acid-soluble iron from the pyrite with HCl treatment decreased the bacterial oxidation rate. The rate of oxygen uptake was altered from linear to non-linear but upon the addition of ferric ions, the rate of oxygen uptake was restored. The direct contact mechanism requires physical contact between the pyrite particles and the bacteria. Silverman (1967) suggested that the total number of attachments between the particles and bacteria would increase with time and would be reflected by the increasing rate of oxygen uptake as demonstrated in the experiment.

KINETICS AND MECHANISM OF THE BACTERIAL OXIDATION OF PYRITE

Le Roux et al. (1973) studied the kinetics and mechanism of the bacterial oxidation of pyrite in shake-flasks at 30°C using Thiobacillus ferrooxidans. 99.8% of the pyritic iron was dissolved in 12 weeks. An increasing iron concentration inhibited pyrite oxidation.

Bacterial and Lixiviant Generator (PILOT SCALE)

Le Roux et al. (1973) investigated the bacterial oxidation of pyrite in a continuous operation of a pilot scale leaching vessel with a capacity of 84 l. The addition of potassium phosphate had a deleterious effect on the oxidation of pyrite due to a decrease in the bacterial population and ferric iron concentration. The amount of pyrite oxidized per week increased as the pulp density was increased. The maximum amount of pyrite oxidized was 15% per week at 6% pulp density.

Bacteria were attached to the pyrite and not affected by vigorous agitation. Lubrol and sodium pyrophosphate dislodged a small fraction of the bacteria (17.6 x 10^8 bacteria per ml) but inhibited bacterial activity.

BACTERIAL PYRITE OXIDATION: RELEASE OF IRON AND SCANNING ELECTRON MICROSCOPIC OBSERVATIONS

Hiltunen et al. (1981) studied bacterial pyrite oxidation. Their results indicated that a large portion of the iron dissolved from pyrite was colloidal or complexed in the leachate. The chelation was attributed to the presence of organic substances in the medium such as yeast extract added to enhance growth of thiobacilli or organic metabolites that the bacteria excreted into the medium.

Ferric iron constituted the major portion of the iron found in filtrates.

Scanning electron microscopy showed the pitted surfaces of pyrite particles in the inoculated pyrite samples. Bacterial cells were detached from the pyrite surface by washing. The specificity of bacterial attachment to the pyrite surface was not determined (Hiltunen et al., 1981). Specific orientation and arrangement of the cells was not observed (as by Bennett and Tributsch, 1978) since irregular cavities and pits were present.

Hiltunen et al. (1981) found that tabular and pseudo-cubic jarosite crystals were found on the pyrite. Shaking enhanced bacterial pyrite dissolution. More pyrite was dissolved with previously adapted bacteria.

EFFECT OF PULP DENSITY ON METAL LEACH RATE AND EXTRACTION DURING THE BACTERIAL OXIDATION OF PYRITE

Atkins (1978) found that the iron leach rates were proportional to pulp density up to approximately 4% (w/v) with an optimum pulp density at 10% (w/v).

Complete metal release from pyrite was obtained within the pulp density range of 2 to 20% (w/v) provided the pH of the suspension was maintained at pH 2.0.

For the bacterial oxidation of pyrite, the optimum cell inoculum was calculated to be 1 x 10^9 cells/ml/1% pulp density (Atkins, 1978). Varying the inoculum size by a factor of 10000 had a negligible effect on the leaching results.

Atkins (1978) leached pyrite samples from different geological origins to completion in the presence of Thiobacillus ferrooxidans at pH 1.0-2.0. Atkins (1978) felt that these results demonstrated that a protective reaction layer on the surface of sulphide particles which accounted for their incomplete oxidation did not form during the oxidation of iron pyrite.

PYRITE DISSOLUTION RELATED TO SURFACE AREA

Paciorek et al. (1981) found that the dissolution of pyrite was directly related to surface area. The rate of soluble iron produced increased as particle size decreased. The chemical process involved in the dissolution of pyrite was unaffected by the ferrous iron concentration since there was no decrease in the rate of soluble iron production with time.

PYRITE OXIDATION BY THIOBACILLUS FERROOXIDANS AND MIXED CULTURES OF BACTERIA

Norris and Kelly (1978b) conducted studies to compare pyrite oxidation by Thiobacillus ferrooxidans with that by mixed cultures of Thiobacillus thiooxidans ATCC 8085, Thiobacillus organoparus NCIB 11170, Thiobacillus acidophilus and

Leptospirillum ferrooxidans; by Thiobacillus TH1; and by uncharacterized natural mixed cultures of acidophilic bacteria.

Leptospirillum ferrooxidans in mixed culture with Thiobacillus organoparus or Thiobacillus thiooxidans or Thiobacillus acidophilus was capable of releasing soluble iron from pyrite. In unmixed pure cultures, none of the above microorganisms were able to degrade pyrite. Leptospirillum ferrooxidans and one of the sulphur-oxidizers must be present for the degradation of pyrite.

Pyrite solubilization by a mixed culture of Leptospirillum ferrooxidans and Thiobacillus thiooxidans was equal to that of natural, mixed cultures but more extensive than that of pure Thiobacillus ferrooxidans strain or Thiobacillus TH1 when grown at 30°C. Norris and Kelly (1978b) suggested that the capacity of mixed cultures to maintain a high rate of pyrite breakdown may be a result of their adaptation to conditions arising during growth on pyrite rather than a greater primary ability to attack the pyrite crystal lattice.

ADAPTATION OF BACTERIA TO PYRITE WITH YEAST EXTRACT

Norris and Kelly (1978b) concluded that the beneficial effect of yeast extract in the adaption of bacteria to growth on pyrite resulted from a wetting effect which enabled the bacteria to adhere to the substrate rather than from a supply of growth factors.

The iron oxidation step appeared to be the growth-rate-limiting step to Thiobacillus ferrooxidans growing on pyrite (Norris and Kelly, 1978b).

SECTION I.4

LEACHING OF METALS

CYCLIC LEACHING PROCESS EMPLOYING IRON-OXIDIZING BACTERIA

Zimmerley et al. (1958) patented a cyclic process for the extraction of metallic components of metallurgical material. The minerals are leached by a ferric sulphate, sulphuric acid lixiviant solution containing motile, non-spore forming, iron-oxidizing, autotrophic, rod bacteria which are tolerant to the metallic ions to be extracted. The spent ferrous iron solution is regenerated to ferric iron in a bacterial regeneration reservoir. Zimmerley et al. (1958) claim that this process is effective for the extraction of copper from copper-bearing pyrite, from mixed oxide and sulphide copper ores, and from concentrates of molybdenite-copper minerals; for the extraction of zinc from zinc-bearing sulphide materials; and for the upgrading of high iron content chromite or titanium-bearing materials.

LEACHING OF CADMIUM SULPHIDE

Pure cadmium sulphide was leached by Thiobacillus thiooxidans (ATCC 8085) in the presence of finely divided sulphur (Brissette et al., 1971). Maximum cadmium extraction occurred at 32°C under the experimental conditions. The activity of the bacteria increased as the pH decreased. The rate of extraction was found to decrease with time and became insignificant after 5 weeks of leaching.

LEACHING OF CHROMITE ORE

A chromite ore was leached by dilute H_2SO_4 generated by Thiobacillus thiooxidans from sulphur (Ehrlich, 1983). In 42 days, 2.7% Cr, 5.6% total Fe and 50% Fe^{2+} were extracted from chromite (5% pulp density) by a stationary culture. Agitating the ore increased the percent extraction. Leaching of autoclaved ore produced similar results.

Chromium and iron extraction generally stopped after 30 to 40 days. The major portion of chromium and iron, however, was not extracted, possibly due to residual amounts locked in the ore. Aluminum and silica were also extracted.

LEACHING OF TETRAHEDRITE

A sample of tetrahedrite containing 8.2% copper and 3076 ounces silver per ton was leached by Thiobacillus ferrooxidans in shake flasks (Duncan, 1967). After 10 days of leaching, 100% of the copper had been solubilized. In the sterile control, 19% of the copper had been extracted in the same time period. No solubilized silver was detected.

LEACHING OF STANNITE

A sample of stannite, containing 3.1% copper and 3.9% tin was leached by Thiobacillus ferrooxidans (Duncan, 1967). After 30 days of leaching, 47.5% of the copper was solubilized compared to 3.0% in the sterile control. Only 12% of the tin was extracted compared to 4% in the sterile control.

LEACHING OF A COPPER-NICKEL SULPHIDE MINERALIZATION

Two samples of a copper-nickel sulphide were leached by Thiobacillus ferrooxidans (Duncan, 1967). Sample 1 contained 1.4% copper, 0.5% nickel and 34.5% iron. After 189 hours of leaching, 75% of the copper and 55% of the nickel was extracted. In the sterile control, 32% of the copper and 25% of the nickel was solubilized. Sample 2 contained 1.3% copper, 1.0% nickel and 32.8% iron. After 189 hours of leaching, 38% of the copper and 53% of the nickel was extracted in the presence of the bacteria compared to 17% of the copper and 21% of the nickel in the sterile control.

LEACHING OF MOLYBDENITE

A thermophilic, chemoautotrophic microbe leached molybdenite and tolerated soluble molybdenum at 60°C (Brierley, C.L., 1974). Sulphur oxidation by the microbe was slightly inhibited by 1000 ppm hexavalent molybdenum but the organism tolerated up to 2000 ppm hexavalent molybdenum. Growth still occurred when the soluble molybdenum concentration was 750 ppm.

A 98.5% molybdenite concentrate was leached at a maximum rate of 6.6 mg Mo solubilized/l/day for 30 days with a 3.3% molybdenum recovery. This rate of solubilization was increased with the addition of yeast extract (0.02%) and 1% iron (II) sulphate. Leaching of the molybdenum in batch reactors produced less soluble molybdenum than leaching in a reactor that had the suspending medium replaced every one or two weeks. This was attributed to the build-up of inhibitory products in the suspending medium in the batch conditions.

The addition of sucrose, lactose, mannitol, alpha-ketoglutaric acid and yeast extract enhanced the leaching of the concentrate; however, a combination of the compounds were not additive in their enhancement. The highest leaching rates were obtained when Fe^{2+} was added in combination with either yeast extract or sucrose. Molybdenite concentrate oxidation was inhibited by sulphur. Solubilization of molybdenum was not influenced by pyrite until the pyrite concentration exceeded 50% of the total solid substrate present.

High-grade molybdenite ore was also leached successfully by the high-temperature microbe. Approximately 40 to 90 ppm molybedenum was leached in 30 days from an ore containing 7.3% molybdenum.

Waste molybdenite material and tailings were not successfully leached by the microbe under the given experimental conditions. The presence of acid-consuming gangue in the waste material increased the pH above the optimum (pH 2-3) for the growth of the microbe. Ferric iron was also precipitated.

OXIDATION OF SULPHIDES BY THIOBACILLUS FERROOXIDANS

Torma and Sakaguchi (1978) investigated the oxidation of analytically pure heavy metal sulphides by a strain of Thiobacillus ferrooxidans. The metal sulphides used were cadmium sulphide (CdS), cobalt sulphide (CoS), cupric sulphide (CuS), cuprous sulphide (Cu_2S), nickel sulphide (NiS), and zinc sulphide (ZnS).

The maximum rate of metal dissolution was found to be directly proportional to the surface area of the metal sulphide. The order of the rate of metal dissolution established for the substrates used in the experiments was: $NiS > CoS > ZnS > CdS > CuS > Cu_2S$. Upon further investigation of this order of the maximum rate of metal dissolution, the researchers suggested that there was a relationship between the rate of metal dissolution and the solubility products of the metal sulphides. Mathematically computed data indicated that the metal sulphide with the highest solubility product had the highest rate of oxidation by Thiobacillus ferrooxidans. Torma and Sakaguchi (1978) felt that the direct relationship between the solubility product and rate of metal sulphide oxidation was because the bacteria remained close to the surface of the substrate in order to have access to the solubilized substrate the moment it became available in the oxidation process. The researchers proposed that the dissociation of the metal sulphides is the first step in microbial oxidation of metal sulphides.

STIBNITE - AN ANTIMONY-BEARING SULPHIDE MINERAL

The optimum leaching conditions for the leaching of stibnite minerals were determined using a stibnite-adapted strain of Thiobacillus ferrooxidans (Torma and Gabra, 1977). The optimum temperature, pH and pulp density were found to be 35°C, 1.75 and 12 g solid substrate per 100 ml of basal salts medium, respectively. A temperature increase of 10°C more than doubled the rate of biological oxidation of stibnite.

The yields of dissolved antimony were approximately 5 to 7 times greater with a leach solution containing Thiobacillus ferrooxidans than with a sterile control. The highest concentration of dissolved antimony was ca 1400 mg/L. Insoluble antimony oxide sulphates, $(SbO)_2SO_4$ and $(SbO_2)_2SO_4$, precipitated on the surface of the stibnite and inhibited bacterial action. Between 55 and 65% of the stibnite was converted to the antimony sulphates.

LEACHING OF METALS FROM SEWAGE SLUDGE

Schönborn and Hartmann (1978) examined the use of bacterial leaching for the separation of metals from digested sewage sludge. Thiobacillus thiooxidans was capable of oxidizing sulphur in an aerated medium of 92.5% digested sludge and 7.5% inoculum. This oxidation decreased the pH value of the sludge from 5.6 to 1.0 to 1.5. Metal sulphides were solubilized as metal sulphates as a result of the acidification of the medium.

Thiobacillus ferrooxidans oxidized metal sulphides in the presence of limited sludge additions (approximately 6%) if the pH was adjusted to 2.5. When Thiobacillus thiooxidans and Thiobacillus ferrooxidans were mixed together with sludge and 1% sulphur, Thiobacillus thiooxidans acidified the sludge and Thiobacillus ferrooxidans oxidized the iron, copper, zinc, nickel and cadmium sulphides.

Heavy metals that exist in a reduced sulphide form in aerobically digested sewage sludge can be bacterially leached by Thiobacillus ferrooxidans (Henry and Wong, 1984). Laboratory scale leaching experiments conducted at the Environmental Engineering Laboratory at the University of Toronto achieved good metal extraction efficiencies: cadmium, 86%; copper, 65%; nickel, 78%; and zinc, 87%. Current experiments involve the determination of the optimum leaching conditions.

SECTION 1.5

COPPER

COPPER

Approximately 5% of the world copper production and 15% of the U.S. copper production is based on the solubilization and leaching of the metal from low-grade ores by Thiobacillus ferrooxidans (Maiouf, 1971; Atkins, 1978, respectively).

Copper can be economically recovered from mine waste that contains 0.25 to 0.5% copper by bacterial leaching (Fletcher, 1970). Low grade ore is irrigated with water or leach liquer acidified with sulphuric acid. The water percolates through the dump and the copper is dissolved. The solution is passed over scrap iron where copper powder is precipitated by the cementation reaction:

$$Cu^{2+} + SO_4^{2-} + Fe(metal) \longrightarrow Cu(metal) + Fe^{2+} + SO_4^{2-}$$

Chalcopyrite or chalcocite are the principle sulphide minerals in most leaching dumps. There may be minor amounts of bornite, covellite and cenargite present; pyrite is a common accessory mineral. Malachite, azurite and chrysocolla are the oxidized copper minerals that may be present

Torma and Banhegyi (1984) stated that it is generally considered that the microbial leaching processes for extracting copper from low-grade ores could produce copper at approximately one third to one half of the cost of the conventional smelting techniques from sulphide concentrates.

Microorganisms used to leach copper ore (Zajic, 1969c)

1) Thiobacillus thiooxidans
2) Thiobacillus ferrooxidans
3) Ferrobacillus ferrooxidans
4) Thiobacillus concretivorus

5) Thiobacillus neopolitanus

Microbial leachability of copper ores

The principal copper ore minerals are pyrite and chalcopyrite (Sirel, 1949 cited by Zajic, 1969c). Sphalerite, millerite, pyrrhotite, pentlandite, cobaltite, arsenopyrite, and magnetite are accessory minerals.

Chalcocite, chalcopyrite, covellite, enargite, bornite and digenite all contain a sulphide moiety. These copper-containing minerals can be leached as the sulphide is oxidized to sulphuric acid. Chalcopyrite and bornite also contain ferrous iron which can be oxidized by leaching bacteria. The arsenic in enargite also offers a second line of attack for biochemical solubilization. Brochantite, antbrite and atacamite are more difficult to leach. This may be due to their toxicity to biological systems. Chrysocolla is also difficult to leach because it is a silicate of copper.

PREDICTED MICROBIAL LEACHABILITY OF COPPER ORES

Chemical composition	Mineral	Moiety oxidized biochemically	Oxidation by Fe^{3+} (a)
Cu_2S	Chalcocite	S_{2-}	+
$CuFeS_2$	Chalcopyrite	S^{2-}, Fe^{2+}	-
CuS	Covellite	S^{2-}	+
$Cu_3(CO_3)_2(OH)_2$	Azurite	-	+
Cu_2O	Cuprite	-	+
Cu_3AsS_4	Enargite	As^{2+}, S^{2-}	-
$Cu_4(OH)_6SO_4$	Brochanite	-	-
$Cu_3(OH)_4SO_4$	Antbrite	-	-
$Cu_2Cl(OH)_3$	Atacamite	-	-
$Cu_2CO_3(OH)_2$	Malachite	-	+
Cu_5FeS_4	Bornite	Fe^{3+}, S^{2-}	+
Cu_9S_5	Digenite	S^{2-}	+
$CuSiO_3.2H_2O$	Chrysocolla	-	-

(a) $Fe_2(SO_4)_3$

(+) easy

(-) difficult

Source: Zajic (1969c)

Copper extraction and oxygen consumption in the biological leaching of "sulphide" copper minerals.

Mineral	Biological Copper Extraction[a] (%)	Stoichiometric Oxygen Requirement (kg/kgCu)
Chalcopyrite, $CuFeS_2$	45-55	2.14
Bornite, $CuFeS_4$	70-80	0.93
Covellite, CuS	90-100	1.01
Chalcocite, Cu_2S	90-100	0.63

[a] Normal value for -400 mesh material under optimized conditions.

Source: McElroy and Bruynesteyn (1978)

Old copper dumps leached more rapidly than new dumps. Approximately 6% more copper was extracted from the old dump (Aramyan and Voskanyan, 1983).

LABORATORY SCALE LEACHING

LEACHING COPPER WITH PSEUDOMONAS DENITRIFICANS AND PSEUDOMONAS FLUORESCENS

In experiments conducted by Pol'kin et al. (1975), Pseudomonas denitrificans and Pseudomonas fluorescens extracted 1.1 g/l of copper at pH 8.8 in 13 days in the presence of glucose and chemically pure CuS.

BACTERIAL LEACHING OF COPPER AT AN INITIAL ALKALINE pH

Polish low-grade copper ores cannot be leached in strongly acidic conditions with the use of Thiobacillus ferrooxidans since the ores are limestone or sandstone type, characterized by neutral or slightly alkaline pH (Lejczak et al., 1980). A mixed population of sulphur-oxidizing Thiobacilli were isolated from a copper waste dump and were able to leach copper from covellin at an initial alkaline pH of 9.1 (Lejczak et al., 1980). Characteristics of the strains were not given. The maximum leaching rate achieved was 3-4 mg Cu^{2+}/l/hr. With some of the isolated cultures, the pH stabilized at 4.0 to 5.0 during the leaching. Cupric ion concentrations of 3-5 g/l were obtained at pH 4.8.

LEACHING OF COPPER ORES WITH MINE WATER

Nandi and Sant (1978) found that microorganisms played a significant role in the leaching of copper from Malanjkhand copper ores (1.07% Cu, 3.18% Fe). After 38 days of leaching, approximately 19% of the copper from a partially oxidized copper ore was extracted compared to 9% in a sterile control.

Gupta et al. (1977) examined the mine water of the Malanjkhand area as a potential medium for the microbiological leaching of copper ores. Mixed microbial flora from two mine water samples (A and B) were developed, subcultured and preserved for leaching studies. Three copper ores were leached for 21 days at pH 3 by the two mine water samples. The amount of copper leached with sample A was 7-72% copper. Sample B was less effective in leaching and only 5 to 54% of the copper was leached.

LEACHING OF CANANEA ORE

Waste Cananea ore containing 0.38% copper, 2.61% iron and 3.5% sulphur was leached with and without the bacterial activity of Thiobacillus ferrooxidans (Bruynesteyn and Cooper, 1974). Rapid copper leaching occurred at pH 2.8 in shake-flask experiments. In the innoculated shake-flasks, 81% of the copper was extracted as compared to 38% in the sterile control flasks. Lowering the pH had a beneficial effect on copper extraction. At pH 1.8, 89% of the copper was extracted with the bacteria and 50% was chemically extracted in sterile controls.

In column studies containing active cultures of Thiobacillus ferrooxidans leaching was also very rapid. After 12 weeks, 64% of the copper was extracted. The extraction rate then gradually decreased and by 21 weeks, approximately 85% copper had been extracted. After the twenty first week, the extraction rate decreased dramatically and 92.2% extraction was not achieved until after another 36 weeks. Upon termination of the column leaching experiment, the residual ore material was analysed. Little leaching had taken place inside the particles that were greater than ½ inch. This suggested that leachable minerals were situated on the exposed surfaces of ore particles.

EFFECT OF SODIUM CHLORIDE ON LEACHING OF A COPPER ORE

Vorreiter and Madgwick (1982) examined the effect of sodium chloride on bacterial column leaching of a low-grade Bougainville copper porphyry ore. Salt irrigation (0.6M) caused a brief increase in copper recovery for the first 15 days after its introduction into the leach solution (145 g/tonne/15 days). The amount of copper extracted decreased to less than 20 mg/l/day after the initial 15 days. When acid leaching was re-established (0.01 M, pH2), the amount of copper recovered increased to 46 mg/l/day. Low levels of sodium chloride remained attached to ore particles and inhibited any further increase in the copper extraction rate.

Bacterial numbers of Thiobacillus ferrooxidans were reduced to less than 10 cells/ml in 0.6 M NaCl solution compared with 10^2cells/ml in 0.01 M H_2SO_4 solution (pH 2.0). The population was restored to 10^3 cells/ml with the resumption of acid leaching. These results indicate that a pre-adapted, chloride-tolerant Thiobacillus ferrooxidans may have a use in waste ore leaching in a sodium chloride solution.

OXIDATION OF COVELLITE

Bacterial oxidation of metal sulphides has been measured by the production of soluble metal ions, by the consumption of oxygen as determined in Warburg manometers and by the production or lack of production of the sulphate ion (Rickard and Vanselow, 1978). Rickard and Vaneslow (1978) suggested that these methods are limited in application because they can only measure reaction rates averaged over an extended time. These researchers investigated the kinetics and stoichiometry of the bacterial oxidation of covellite (CuS) by Thiobacillus thioparus and Thiobacillus ferrooxidans using a polarographic oxygen probe.

Other samples of ore were covered with a brown, laquer-like precipitate. Chemical analysis showed that iron was the major element and copper, the minor element in both the brown and yellow precipitates. Trace amounts at As, Zn, Pb, Ba, Sn, Zr, Rb and Sr were also detected.

Thiobacillus ferrooxidans was distributed throughout the entire ore bed and was associated with the ore samples and the precipitates. There was an increase in bacterial activity and numbers at the top of the ore column as compared to the bottom of the ore column due to a better availability of oxygen and ferrous iron at the top of the column.

There was also more copper remaining at the top of the ore bed. This was due to the presence of more precipitates at the top of the column.

The average amount of copper extracted over the four year period was 25%.

REMOVAL OF IRON PRECIPITATES ON ORE SURFACES DURING COPPER LEACHING BY T. FERROOXIDANS

The bacterial leaching by Thiobacillus ferrooxidans of a low-grade quartz feldspar porphyry copper ore was studied in laboratory percolation leaching columns over a 3 year period to simulate waste dump events (Madgwick and Ralph, 1981). After a year of leaching, the rate of copper recovery decreased accompanied by a build-up of ferric iron precipitates (goethite) on the ore particle surfaces. Scanning electron microscopy with elemental analysis and electron probe analysis indicated that extensive copper oxide was present in the iron precipitate. Madgwick and Ralph (1981) suggested that these iron precipitates formed because there was an insufficient acid supply at pH 2 to maintain the ferric iron in a soluble form and to balance the consumption by gangue material present in the ore. The researchers assumed that the decrease in copper release was due to the flocculated oxides and hydroxides of iron which shielded the surfaces of the ore particles from further bacterial oxidation by Thiobacillus ferrooxidans. A high acid cycle (pH 1.5) removed the iron oxides in 100 days. Copper release increased temporarily but then declined steadily. Bacterial numbers increased and remained elevated for the duration of the leaching studies. Since these conditions should favour increased leachability of chalcopyrite, the researchers suggested that the bacteria did not attack the substrate as effectively in the more acid environment.

COPPER LEACHING IN INDIA

Approximately 366 million tons of the reserve copper in India is of the low-grade variety (1% or less copper) (Rangachari et al., 1978). Ten different low-grade copper ore samples with copper contents ranging from 0.2% to 1.5% were leached in mine water containing Thiobacillus ferrooxidans and Thiobacillus thiooxidans at pH 2.0 - 2.5 in shake-flasks at 28°C. The different ore samples varied in leachibility by 20 to 40%. Within 90 days, 40-80% of the copper was leached from the ores.

LEACHING OF CHALCOPYRITE

Chalcopyrite is a major source of copper in Canada. Bioleaching by Thiobacillus ferrooxidans can cause this mineral to undergo oxidation, as indicated by the following equation:

$$2CuFeS_2 + 8\tfrac{1}{2}O_2 + H_2SO_4 \rightarrow 2CuSO_4 + Fe_2(SO_4) + H_2O$$

(Sakaguchi et al., 1976).

Groudev (1980a) found that the presence of bacteria increased the chalcoyrite electrode potentials as well as the difference between the electrode and oxidation reduction potentials. This accelerated the copper extraction rates. Yukawa et al. (1978) developed a kinetic model for the bacterial leaching of chalcopyrite ($CuFeS_2$) which takes into account the chemical and microbial reactions that can occur under acid conditions. Based on the stoichiometries for chalcopyrite leaching that have been reported in the literature, the model provides a basis for the analysis and quantification of a leaching system.

Razzell and Trussell (1963) investigated the bacterial leaching of chalcopyrite by Thiobacillus ferrooxidans. They found that copper was released more rapidly from fine particles than from coarse ones. Approximately 3 times more copper was leached from particles of a size less than 325 mesh in 7 days than from particles of 70 to 140 mesh in 58 days. Low phosphate levels decreased the rate of copper and iron release from the chalcopyrite; high phosphate levels inhibited the release of these ions or precipitated them as insoluble phosphates. They concluded that bacteria were more effective for leaching chalcopyrite than ferric iron alone.

LEACHING CHALCOPYRITE CONCENTRATES

Sakaguchi et al. (1976) used a pure strain of Thiobacillus ferrooxidans to determine the optimum leaching conditions of a naturally-occurring chalcopyrite concentrate. The optimum temperature and pH for copper extraction were 35°C and 2.3, respectively. The copper extraction rate was dependent on the initial pulp density. The optimum pulp density, which is defined as the mass of solid substrate (g) multiplied by 100/volume of the liquid medium (ml), was 22%. This value is slightly lower than the 26.7% optimum pulp density Torma et al. (1979) found for a chalcopyrite flotation concentrate. The amount of copper extracted form the naturally-occurring chalcopyrite varied between 54 and 71% in the solutions innoculated with Thiobacillus ferrooxidans (Sakaguchi et al., 1976). In sterile solutions, the amount extracted was between 13 and 15%. This large difference in the percent extraction between the sterile and inoculated solutions indicates the advantageous effects of microorganisms in the leaching of chalcopyrite.

Pinches et al. (1976) used Thiobacillus ferrooxidans to extract copper from chalcopyrite concentrates obtained from two different locations. The copper yields and leach rates of the two concentrates differed significantly. These differences were attributed mainly to mineralogical factors arising from different geological origins.

Pinches et al. (1976) concluded that the most important variable affecting the amount of copper extracted from the chalcopyrite concentrates was the initial mineral particle size.

Another important factor that affects the ability of bacteria to completely break down the chalcopyrite particles is the formation of layers of insoluble leach products on the mineral surfaces (Pinches et al., 1976). Sakaguchi et al. (1976) and Torma et al. (1979) found that during the leaching of a chalcopyrite concentrate most of the dissolved iron was precipitated as jarosite, a product of partial hydrolysis of ferric sulphate ($Fe_2(SO_4)$). The jarosite precipitated on the surface of the chalcopyrite and inhibited the bacterial action. The studies indicated that the leaching stopped at approximately 60% extraction. Sakaguchi et al. (1976) hypothesized that this problem could be eliminated if the leach material was reground to expose the chalcopyrite. They recovered, wet ball milled and releached, leach residue from a 22% pulp density leach suspension and found that the amount of copper in the final extraction was 80%.

Sakaguchi et al. (1976) and Torma et al. (1979) developed flow sheets for industrial scale microbiological treatment for chalcopyrite concentrates. Although they recognize the many problems involved in scaling up their process to pilot plant and industrial scale, they feel that these process could produce a high rate and yield of copper extraction. Both of the proposed treatments for the chalcopyrite involve the regrinding of the material after each leaching to obtain maximum copper extraction and the recovery of the copper by cementation or by solvent extraction with electrowinning. The leach solution can be recycled. Torma et al. (1979) felt that their microbiological leaching process would not compete economically with existing smelting processes for the treatment of chalcopyrite flotation concentrates unless the concentrate was produced in a remote area and had high transportation costs, or if the mineral deposit was small and did not justify the expensive installation of a conventional smelting plant.

CONTINUOUS BIOLOGICAL LEACHING OF CHALCOPYRITE CONCENTRATES

McElroy and Bruynesteyn (1978) conducted a two year study to determine the technical and economic feasibility of using biological leaching for the extraction of copper from chalcopyrite concentrates. Thiobacillus ferrooxidans was used in the study. The experimental study demonstrated that technically, biological leaching of chalcopyrite concentrates using Thiobacillus ferrooxidans is feasible. However, the economic analysis suggested that the continuous biological leaching process developed in the study would be only slightly competitive with conventional smelting. McElroy and Bruynesteyn (1978) listed the following competitive advantages of the process:

1. Negligible atmospheric emissions
2. Refined copper production (This refined copper may have a lower transportation

cost and increased market flexibility)

3. Small scale mine site operations (min. ~40+pd of concentrate)
4. reduced cost for bornite-, chalcocite-containing concentrates regardless of grade or pyrite content.
5. Increased silver, gold, molybdenite(?) returns in concentrates
6. Production of dilute sulphuric acid (for leaching of oxide ore and for mill tailing and/or acid consuming waste dumps).

As a result of these advantages, it was concluded that leaching may be an option over conventional smelting techniques when at least one of the following factors apply:

1. Severe restrictions on atmospheric emissions from conventional smelting result in significant increases in smelting charges
2. Transportation costs for concentrates increase
3. There is a local market for refined copper
4. Sulphuric acid is needed for local use
5. Concentrates contain bornite, chalcocite,etc. particularly if the content of pyrite is high and/or grade is low
6. Concentrate contains substantial amounts of molybdenite
7. The price of precious metals is high and/or there are high smelter deductions.

LEACHING OF CHALCOPYRITE ORE

Deepak and Das (1981) studied the leaching of copper from a low-grade chalcopyrite ore using Thiobacillus ferrooxidans (NCIB 9490). Optimum temperature and pH for leaching were 35°C and 2.7, respectively. Decreasing the particle size of the ore from 190 to 60 µm increased the percent copper extracted from 21.8 to 63%. Agitation enhanced the rate of leaching.

LEACHING CHALCOPYRITE ORE AT AMBIENT AND ELEVATED TEMPERATURES

Madsen and Groves (1983) investigated the percolation leaching of a chalcopyrite-bearing ore with bacteria at ambient and elevated temperatures. The extraction rate by Thiobacillus ferrooxidans and Thiobacillus thiooxidans for the ambient temperature (25°C) leaching period was very slow. In 1,135 leaching days, only 5.0% of the copper was extracted. This is equivalent to 0.12 kg copper per metric ton of ore per year. The authors suggested that leaching of chalcopyrite produced a thick layer of sulphur that covered the unreacted chalcopyrite and therefore inhibited the microbial leaching activity.

When the temperature of the chalcopyritic ore column and leaching solution was increased to 50°C, the copper extraction rate decreased. The total and ferric iron concentration of the solution also decreased as a result of the decreased solbility of ferric iron at the elevated temperature. Madsen and Groves (1983) concluded that at increased temperatures, bacterial activity decreased and as a result there were insufficient ferric irons present to maintain the copper leaching rate that was attained at an ambient temperature level of 25°C.

The ore column was innoculated with the thermophilic microbe species Sulfolobus acidocaldarius and Sulfolobus brierleyi at 60°C. The copper extraction rate increased to a rate that was 6.64 times greater than the leaching rate attained at ambient temperature. This leaching rate decreased after approximately 120 days of the rapid copper extraction. This decrease was attributed to the diminished supply of readily accessible chalcopyrite mineral particles in the fine ore fraction and on the outermost portion of the larger ore fragments.

Low-grade chalcopyrite copper ore was leached by two strains of acidophilic, thermophilic microbes belonging to the genus Sulfolobus (Brierley, C.L., 1979). These microorganisms, Sulfolobus acidocaldarius and "ferrolobus", oxidize reduced sulphur and iron compounds in the acid pH range at temperatures from 50 to 80°C.

The Sulfolobus and "ferrolobus" enhanced the copper extraction by four to six times. A rest/leach cycle with or without aeration did not influence the overall extraction. Thiobacillus ferrooxidans was relatively ineffective in extracting copper from the ore.

EFFECT OF INTERRUPTED IRRIGATION ON BACTERIAL LEACHING OF CHALCOPYRITE ORE- PILOT SCALE

Grote et al. (1981) studied the effect of interrupted irrigation on copper release and bacterial activity during the leaching of chalcopyrite ore in pilot-scale column leaching experiments. Copper concentrations increased by a factor of about 16 and bacterial iron oxidation by a factor of 3 after a 14 day rest period. The optimum duration of rest periods was between 7 and 20 days. Longer periods led to excessive drying where iron precipitates became irreversibly consolidated and blocked further attack at sulphide oxidation sites. The main practical uses of rest periods are to conserve water and to concentrate dilute copper leachates for more efficient solvent extraction procedures.

LEACHING OF GALLIUM-BEARING CHALCOPYRITE

The following reaction occurs during the leaching of gallium-bearing chalcopyrite by Thiobacillus ferrooxidans (Torma, 1978b):

$$3Fe_2(SO_4)_3 + Ga_2S_3 \longleftrightarrow Ga_2(SO_4)_3 + 6FeSO_4 + 3S^o$$

The gallium sulphide is oxidized by the ferric ions generated by bacterial activity. However, experiments by Torma (1978b) on pure synthetic gallium sulphide indicate that the bacterium is able to oxidize the sulphur in the gallium sulphide complex directly. The following reaction would also take place:

$$Ga_2S_3 + 6O_2 \xrightarrow{bacteria} Ga_2(SO_4)_3$$

The highest leaching activity occurred with high grade chalcopyrite and gallium-bearing chalcopyrite concentrate. The highest dissolved gallium and copper concentrations obtained with the gallium-bearing chalcopyrite concentrate were 2.2 and 40.2 g/L, respectively, at 25% pulp density. The amount of gallium and copper extracted in sterile solutions were only 8 to 15% of the above.

The lowest leaching activity occurred on synthetic gallium sulphide (Ga_2S_3) while intermediate activity occurred on pyrite, covellite, and chalcocite.

Madgwick et al. (1981) examined typical viable cell counts of acidophilic bacteria and sulphur oxidizers in leachates of percolation columns and ore dumps. Pilot-scale laboratory columns always had higher total cell counts.

They found that it was possible to determine the stage of biodegradation of a copper porphyry chalcopyrite/pyrite from a description of the relative abundance of bacteria in leachates. Heterotrophic species were the first inhabitants in a fresh ore body and only the heavy metal tolerant species survived once leaching started. There was a gradual transition to more acid-producing sulphur-oxidizing species with the ultimate presence of Thiobacillus ferrooxidans.

They also suggest that a major restraint on bacterial growth and the subsequent metal extraction is the irregular penetration of acid through an ore body due to consumption of acid by gangue material.

LEACHING OF COPPER - LABORATORY AND PILOT SCALE

LEACHING OF A CARBONATE-RICH GERMAN COPPER SHALE

German copper shale containing about 12 million tons of copper is the most important copper ore in the German Federal Republic (Bosecker et al., 1978). In 1955, conventional mining of the shale was stopped due to unprofitable production costs resulting from low copper content and a thin copper-bearing layer. Bosecker et al. (1978) investigated the feasibility of extracting copper from the shale with bacterial leaching. Laboratory tests were conducted in air-lift percolators (as a model for dump and heap leaching) and in shaking Erlenmeyer flasks. Thiobacillus ferrooxidans and Thiobacillus thiooxidans were used as leaching agents. The pH values in the leach suspensions continuously increased during the course of all the experiments. A loss of bacterial activity accompanied the pH increase. The increase in pH was attributed to the high carbonate content of the copper shale. The researchers concluded that bacterial leaching of the shale was feasible only after the previous neutralization of the carbonate or with the simultaneous addition of sulphuric acid.

In pilot plant units of glass-fibre columns, the effluent leaching suspension was aerated to accelerate the bacterial oxidation of ferrous iron to ferric iron. An oxygen demand of 37.2 kgO_2 per ton of copper shale was necessary for the bacterial oxidation of metal sulphides and without the artificial aeration, several years would have been needed to transport sufficient oxygen through the ore. The best extraction rates were obtained with a partial neutalization of the carbonate to pH 2. Approximately 22% of the copper and 55% of the zinc was extracted from the ore in 245 days. The average extraction rate was 250 mg Cu/hr per ton of copper shale and 560 mg Zn/hr per ton of copper shale. Bosecker et al. (1978)

estimated that 70% of the copper would be extracted in 3 years and 80% of the zinc would be extracted in 1½ to 2½ years.

BENCH SCALE LEACHING

LEACHING OF A COMPLEX COPPER-ZINC SULPHIDE ORE

A complex sulphide ore containing 4% copper and 8% zinc was leached bacterially using Thiobacillus ferrooxidans (Parsons, 1975a). After 43 weeks of leaching ore in a bench-scale percolation leaching apparatus containing Thiobacillus ferrooxidans, 58% of the copper and 57% of the zinc was extracted. This was equivalent to 17 lbs. of copper and 34 lbs. of zinc extracted for every ton of ore treated.

Parsons (1975c) felt that these results warranted further testing on a tonnage scale using a minus 8 inch ore that would be used in commerial applications. Two and a half tons of the ore, containing 5.5% copper and 8.6% zinc, was leached in a simulated vertical stope with an acidic ferric sulphate solution containing Thiobacillus ferrooxidans. After 24 successive leaches over a 126 week period, 31.1% of the zinc and 30.4% of the copper were extracted. Approximately 38 lbs. of copper and 60 lbs. of zinc were extracted per thousand tons of ore treated per day. The original iron content of the leaching solution had no effect on the rate of copper and zinc dissolution.

SCALE-UP FOR COPPER EXTRACTION

Bruynesteyn and Cooper (1974) provided evidence indicating that simple laboratory shake-flask tests can be used to predict the amount of copper that can be extracted from leaching dumps containing ores similar to the type tested. Experiments performed in shake-flasks, six inch diameter columns and in test dumps by these researchers gave similar results for the amount of copper extracted from Cananea ore. Shake-flasks and column tests gave copper extractions of 89 and 92%, respectively. Copper extraction for the test dump was 80.6%. The rate of copper extraction, however, did vary significantly because of the different particle sizes. In the shake-flask tests at pH 1.8, 80% copper extraction was achieved in about 1.8 weeks. In column studies using -2 inch material and in dump studies using run-of-the-mine waste rock, 80% extraction was reached in 18½ weeks and 97 weeks, respectively. For the Cananea ore tested, shake-flask rates were approximately 10 and 54 times faster than those achieved in columns and leach dumps, respectively.

LARGE SCALE TEST FACILITIES

Murr and Brierley (1978) leached approximately 1.7 x 10^5 kg of waste copper-bearing rock in insulated stainless steel containers. These large-scale test facilities allowed vertical temperature profiles in the ore bodies to be correlated with bacterial population (most probable number, MPN, of _Thiobacillus ferrooxidans_), and oxygen distribution and consumption. No bacterial catalysis of any consequence occurred until there was at least 10^5 organisms/g sample in the waste body. Oxygen consumption occurred when MPN reached 10^7 cell/g. Exothermic pyrite oxidation produced temperatures within the orebody that occasionally reached 55°C. _Thiobacillus ferrooxidans_ populations declined at the high temperatures. However,

O_2 consumption increased indicating that a thermophile may exist at higher temperatures.

Murr and Brierley (1978) suggested that bacteria may limit dump temperatures via a feedback mechanism which regulates the rate of catalysis in order to regulate the temperature to a range where their ability to catalyze would be a maximum. It was proposed that advancing the temperature regime by inoculating with a higher-temperature microbe may increase copper leaching considerably.

In 1983, B.C. Research (Vancouver) developed a low-cost copper extraction process that potentially could eliminate smelting or simplify a smelter's environmental problems. The new technique involved a biological leaching step that selectively extracted 95% or more of the copper from chalcopyrite, inhibited oxidation of pyritic sulphide and yielded elemental sulphur as a by-product. Thiobacillus ferrooxidans was used to convert the copper to its soluble form (Short and Parkinson, 1983).

UNDERGROUND COPPER LEACHING

Lung (1984) generated an acidic ferric leaching medium by the bacterial oxidation of pyrite under commercial leaching conditions in an underground copper mine. This bio-generation of a ferric leaching medium may be useful if an ore body does not have a sufficient pyrite content for leaching to take place. Experiments were conducted at the Chin-Kua-Shih Copper Mine, an underground mine in Taiwan, where enargite is the principal copper mineral. In certain ore bodies the pyrite content is insufficient for economical leaching operations. An abandoned underground mining shaft was converted into a lixiviant generation column. The materials used for the ferric lixiviant generation included Thiobacillus ferrooxidans and Thiobacillus thiooxidans, a pyrite concentrate from the mine's ore beneficiation plant, industrial grade sulphuric acid and untreated silica sand. The average temperature during the experiment was 18°C. At this temperature, underground culturing of the thiobacilli under continuous aeration was successful. The results of the experiment indicated that the bacteria accelerated the oxidation of pyrite when compared to the rate of oxidation obtained using sulphuric acid alone. The bacteria released sufficient quantities of ferric iron into the leach solution to extract copper from the minerals.

MICROBIAL STUDIES IN COPPER-PYRITE DEPOSITS

Karavaiko et al. (1974) conducted microbiological studies in the copper-pyrite deposits of the Southern Urals. They found that Thiobacillus ferrooxidans, Thiobacillus thiooxidans and Thiobacillus thiocyanoxidans were most widespread in finely pulverized ores. The oxidation of the sulphide minerals and leaching of the metals were also most active in the finely pulverized ores indicating that there was a relationship between the oxidation activity of the bacteria and accessible mineral surface area. The most important factors that limited the bacterial activity in ore water was the varying salt concentration and low temperatures (8 to 12°C).

FEASIBILITY OF COMBINING EXTRACTION-SORPTION TECHNOLOGY OF SOLUTION TREATMENT WITH BACTERIAL LIXIVIATION OF COPPER-ZINC ORES

Golomzik et al. (1980) investigated the influence of extraction of copper and sorption of zinc on the activity of Thiobacillus ferrooxidans in circulating solutions. The concentration of bacteria was 10^7 cells/ml in a lixiviant solution prior to copper extraction. After the extraction of copper the concentration of bacteria decreased to 10^4 cells/ml and their activity decreased 3 fold. Activity of the cells fell to zero within a week. Prior to zinc sorption, the number of bacteria in the pulp tailings was 10^3 cells/ml. After the pulp had settled, no cells were found in the clarified solution. Bacteria must be added to the clarified solution recirculated for lixiviation.

ENHANCED BACTERIAL LEACHING OF METALS WITH CARBAMIDE PHOSPHORIC ACID

O'Connor et al. (1972) patented a process for the extraction of copper, zinc, nickel, cobalt, uranium, vanadium, molybdenum, titanium or chromium from metallurgical materials. A sulphate lixiviant solution containing either Thiobacillus ferrooxidans or Thiobacillus ferrooxidans and Thiobacillus thiooxidans and carbamide phosphoric acid was brought into contact with the metallurgical material. O'Connor et al. (1972) claimed that the addition of carbamide phosphoric acid in the lixiviant solution increased the amount of metal leached. For example, the amount of copper leached over a 96 hour period was increased by 30% using a solution containing carbamide phosphoric acid.

The initial leaching rate of chalcocite by Thiobacillus ferrooxidans and Thiobacillus thiooxidans was also enhanced by the addition of carbamide phosphoric acid (Jacobson et al., 1973); however, it was concluded that with time, the total amount of copper leached would be the same in leaching columns with or without the nutrient. The initial increase in leaching rates may be important in a commercial leaching operation where the lixiviant passes quickly through a dump. The concentration of soluble copper may be increased due to the increased bacterial activity.

SECTION I.6

URANIUM

URANIUM

The most common primary uranium mineral is uranite (UO_2) (also known by the varietal name of pitchblende). Brannerite, davidite, caffinite and carnotite are other known uranium minerals. The minerals are concentrated in pegmatites, hydrothermal veins, sandstones and black shales. The hydrothermal veins are the deposits of main economic importance.

Uranium ores do not exist as sulphides. They usually occur as insoluble oxides (Lundgren and Silver, 1980). Uranium has four oxidation states (+3, +4, +5 and +6). The tetravalent and hexavalent forms are predominant in ore materials and as environmental contaminants (Tuovinen and Dispirito, 1984). Hexavalent uranium is highly soluble as UO_2^{2+} in acid solutions and as carbonate, phosphate and sulphate complexes in neutral solutions.

Uranium solubilization involves the oxidation of the tetravalent form to the hexavalent uranyl ion (Soljanto and Tuovinen, 1980). Uranite (UO_2), for example, can be solubilized by oxidation in dilute sulphuric acid or in acidic ferric sulphate as indicated by the following reactions:

$$UO_2 + 2Fe^{3+} \longrightarrow UO_2^{2+} + 2Fe^{2+}$$

$$UO_2 + Fe_2(SO_4)_3 \longrightarrow UO_2SO_4 + 2FeSO_4$$

$$UO_2 + 2H^+ + \tfrac{1}{2}O_2 \longrightarrow UO^{2+}{}_2 + H_2O$$

$$UO_2 + H_2SO_4 + \tfrac{1}{2}O_2 \longrightarrow UO_2SO_4 + H_2O$$

Uranium leaching can be enhanced in the presence of iron-and sulphur-oxidizing thiobacilli. These microorganisms can regenerate acidic ferric sulphate ($Fe_2(SO_4)_3$) from ferrous sulphate ($FeSO_4$) which is produced in chemical leaching and they can oxidize consitituent ore materials such as pyrite and other sulphide minerals to ferric iron (Fe^{3+}) and sulphuric acid.

$$2Fe^{2+} \longrightarrow 2Fe^{3+} + 2e^{-}$$

$$2Fe^{2+} + 2H^{+} + \frac{1}{2}O_2 \longrightarrow Fe_2(SO_4)_3 + H_2SO_4$$

$$2FeS_2 + H_2O + 7\frac{1}{2}O_2 \longrightarrow Fe_2(SO_4)_3 + H_2SO_4$$

Tuovinen and Dispirito (1984) reported that uranium (as either U^{4+} or UO_2) is oxidized directly by Thiobacillus ferrooxidans when iron is absent (<0.5 µM). When soluble iron is present, uranium is oxidized by ferric iron. The ferrous iron formed after this oxidation reaction is then reoxidized to ferric iron by Thiobacillus ferrooxidans. The end products are Fe^{3+} and UO_2^{2+} which inhibit both oxidations.

Ivarson (1980) found that in the presence of nonautoclaved Thiobacillus ferrooxidans cells, U^{4+} was oxidized at a greater rate than that occuring with chemical oxidation. The oxygen uptake associated with U^{4+} oxidation was accelerated by the bacteria. This may have been the result of cyclic regeneration of residual amounts of ferric iron in the cells.

Other metals in uranium-containing ore materials can also be solubilized during the leaching of the ore in acid conditions (Tuovinen and Dispirito, 1984). Thorium as well as uranium was leached from uranyl thorianite ($Th(U)O_2$) containing ore material in the presence of an enriched population of iron-oxidizing thiobacilli.

MICROBIAL LEACHING OF URANIUM METALS

Uranium material[a]	Chemical composition	Predicted degree leaching with microbes[b]
Uranite	UO_2	+
Hydrous oxides		
Gummite	$UO_3.nH_2O$	+
Bacquerelite	$2\ UO_3.3\ H_2O$	+
Multiple oxides		
Brannerite	$(U,Ca,Fe,Th,Y)_3Ti_5O_{16}$	+
Davidite	$(Fe,Ce,U)(Ti,Fe,V,Cr)_3 (O,OH)_7$	+
Silicates		
Coffinite	$U(Si_4)_{1-X}(OH)_{4X}$	-
Hydrous silicates		
Uranophane	$CaO.2UO_3.2SiO_2.6H_{2+O}$	+-
Beta-uranotile	$CaO.2UO_3.2SiO_2.6H_2O$	+-
Sklodowskite	$MgO.2UO_3.2SiO_2.6H_2O$	+-
Hydrous phosphates		
Autunite	$CaO.2UO_3.P_2O_5.8H_2O$	+
Torbenite	$CuO.2UO_3.P_2O_5.8H_2O$	+
Uramphite	$NH_4(UO_2)(PO_4).3H_2O$	+
Hydrous arsenates		
Zeunerite	$CuO.2UO_3.As_2O_5.8H_2O$	+
Carnotite	$K_2O.2UO_3.V_2O_5.3H_2O$	+-
Tyuyamunite	$CaO.2UO_3.V_2O_5.8H_2O$	+-
Hydrous sulphates		
Zippeite	$2UO_3.SO_3.n\ H_2O$	+

Uranopilite	$6UO_3.SO_3.n\ H_2O$	+
Johannite	$CuO.2UO_3.2SO_3.7H_2O$	+
Carbonates		
Schroeckingerite	$3CaO.Na_2O.UO_3.CO_2$ $.SO_3F.10H_2O$	+
Organic compounds		
Urano-organic complexes		+
hydrocarbons		
Thucholite(Carburan)		

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[a] Bittus (1963) cited by Zajic (1969e)

[b] Easy,+; hard,-; variable,+-

LEACHING OF URANIUM BY THIOBACILLUS FERROOXIDANS

The continuous leaching of uranium by Thiobacillus ferrooxidans was investigated by Tomizuka et al. (1976) using a single stage, continuous culture apparatus. Optimum temperature for the specific growth rate of Thiobacillus ferrooxidans was between 29 and 34°C; optimum pH was between 2.3 and 2.7. Optimum aeration rate was greater than 5 x 10^{-7}g mole O_2/ml.min. atm. Under these conditions, the maximum number of cells occurred in approximately 42 hours. The doubling time was 5.4 hours.

Experiments on the continuous leaching of uranium was conducted on the ferrous sulphate medium with 10% ore. The pH and temperature were 2 and 30°C, respectively. Over 80% of the uranium was leached by operating the continuous leaching at a dilution rate of 0.011 hr^{-1}.

EFFECT OF pH ON LEACHING A PYRITIC URANIUM ORE

Bruynesteyn and Vizsolyi (1981) conducted a series of experiments to evaluate the effect of pH and Eh on the chemical and bacterial leaching of a low grade uranium ore. The pH in general had less effect on the biological leaching process when compared to its effect on chemical ferric iron leaching. For example, at pH 1.6 the biological leach gave 100.6% uranium extraction and the chemical ferric iron leach 69.8% extraction. The authors attributed this difference between the two leaching systems to rapid bacterial break down of pyrite and concluded that approximately 30% of the uranium in the ore was associated with the pyritic materials. They suggested that a biological leach should be used for heap or dump leaching of a pyritic ore and that the heap or dump should be constructed in such a way that optimum conditions for bacterial growth would be maintained. Any conditions that limited bacterial activity would revert the leaching process back to the less efficient chemical ferric iron leach.

After the leaching tests were terminated, the ore in the columns was washed twice with wash water of the appropriate pH. These washings produced significant amounts of uranium (10-15%), indicating that any residual waters in a commercial leach dump or heap operation using finely sized material would contain high uranium concentrations. The authors recommended that any dump or heap leach operation should have a counter current washing operation to extract the uranium contained in residual waters.

LEACHING OF PYRITIC URANIUM ORES

Brierley (C.L., 1978a) feels that bacterial leaching of pyritic uranium ores is both feasible and economical for the ores that do not have a low pyrite content. During studies of bacterial leaching of a low-pyrite-content uranium ore using Thiobacillus thiooxidans (ATCC 8085), Thiobacillus ferrooxidans (ATCC 19859), Sulfolobus acidocaldarius and a Sulfolobus-like organism, Brierley (C.L., 1978a) found that acid consuming material, a lack of energy source, and a slow development or activity of the bacteria within the ore indicated that bacteria could not be used effectively for the leaching of uranium ores that have a low pyrite content.

LEACHING OF A CARBONATE-CONTAINING URANIUM ORE

Bosecker and Wirth (1980) studied the applicability of bacterial leaching of sedimentary uranium ore. After 120 days of leaching, 26.9% of the uranium had been extracted from the ore in percolator columns. However, Thiobacillus ferrooxidans was only active after the carbonate content of the ore had been neutralized. Thiobacillus thiooxidans extracted 5.4% uranium during the same time period. Leaching using a mixture of both strains of thiobacilli resulted in 11.5% uranium extraction.

Thiobacillus ferrooxidans extracted 92% uranium, in 20 days in the shake flask experiments. Thiobacillus thiooxidans recovered 11% in the same time period. After 40 days of leaching, mixed cultures of both strains extracted 90% uranium.

The shake flask and percolator column experiments indicated that this ore was amenable to bacterial leaching if the pH was maintained in a range suitable for the activity of Thiobacillus ferrooxidans. The carbonate in the ore was sufficiently neutralized when 60-80 kg H_2SO_4 per 10^{-6}g uranium ore was supplied. The researchers suggested that Thiobacillus thiooxidans could produce the acid to save H_2SO_4 expense since the pH decreased during the growth of these bacteria. Thiobacillus ferrooxidans would be used to extract the uranium. Bosecker and Wirth (1980) found that the pulp density affected bacterial carbonate neutralization. At 15% pulp density, bacterial acid production only lowered the pH to 3.5 and sulphuric acid had to be added. When the pH dropped to 2.0, 97% uranium was extracted in four days by Thiobacillus ferrooxidans. At 20% pulp density, only 3% uranium was extracted. This was attributed to the inhibition of Thiobacillus ferrooxidans due to the increased concentrations of soluble ore components (K^+, Mg^{++}, Na^+) during carbonate neutralization. The researchers described a counter-current vat leaching process that successfully avoids the accumulation of toxic compounds during uranium leaching.

LEACHING OF A WASTE URANIUM MATERIAL

Barbic et al. (1976) studied the bacterial leaching of a uranium ore from which 70-80% of the uranium had been previously leached by chemical hydrometallurgical processes. When only Thiobacillus thiooxidans was added to the leaching column, 39.9% of the uranium was leached from the ore. With a mixture of Thiobacillus ferrooxidans and Thiobacillus thiooxidans, 63.4% of the uranium was extracted compared to 20.5% in the sterile control. The highest consumption of sulphuric acid was found in the control system (53.6 g/g uranium leached as compared to 16.1 g/g uranium leached when both bacterial strains were used).

In another series of experiments, 11.53 g/l sulphuric acid was added to the leaching experiments at the beginning of the experiments. More than 60% of the uranium was extracted into solution after 60 days. The authors felt that bacterial leaching could be used economically in the treatment of waste uranium material in a short period of time that otherwise could not be extracted by conventional procedures.

BACTERIAL LEACHING OF A PRELEACHED URANIUM ORE

Zajic and Ng (1976) found that the use of ores preleached with steam to remove readily solubilized uranium for bacterial leaching studies produced more reliable and reproducible results. Over 98.5% of U_3O_8 was leached in 9 days at pulp densities of 0.2 g/ml and 0.4 g/ml. Higher pulp densities limited bioleaching under the experimental conditions. Optimum bioleaching of a uranium ore (average U_3O_8 content 0.356%) occurred when the O-R potential was maintained at 0.764 v. and pH was 2.0 (Zajic and Ng, 1976).

Zajic and Ng (1976) studied the semi-continuous bioleaching of a 5% pulp density preleached ore. Fifty percent by volume of the leaching system was removed and replaced at 1-, 3- and 5-day intervals. Replacement was made four times at each time interval. On a 1-day basis, 75% of the uranium was recovered after four transfers. On a 3-day transferring interval, 100% of the uranium was solubilized after the fourth transfer while on a 5-day basis, 98.5% of the uranium was leached at the first transfer.

SEMI-CONTINUOUS, LABORATORY SCALE, URANIUM PROCESS PLANT

A laboratory-scale, semi-continuous uranium process plant was described by Manchee (1977). The uranium was leached from an ore by a lixiviant that consisted of ferric sulphate (10 g/l Fe) in sulphuric acid plus small amounts of ammonia, phosphate and magnesium required for bacterial growth. The pregnant liquor was passed through a suitable ion-exchange resin which preferentially adsorbed the uranium. The uranium-free liquor that emerged from the resin column was passed to a stirred and aerated vessel at 30°C containing Thiobacillus ferrooxidans. These organisms oxidized the ferrous iron back to the ferric state. The regenerated lixiviant was then adjusted to the required pH with sulphuric acid and recycled through fresh uranium ore.

The main difficulty with the above process was that the bacteria inconsistently reoxidized the iron in the uranium-free liquor that came from the resin column. This was attributed to adverse effects of ion-exchange resins on bacterial growth. Careful selection of a resin is therefore essential for the success of this proposed leaching plant. A methonal-washed Amberlite Xe 270 resin did not adversely affect the bacterial population and as a result, the ferric iron was regenerated in the leach liquor.

Uranium recovery with this laboratory scale leaching plant exceeded 94% over a period of several months of operation.

BENCH SCALE URANIUM LEACHING

McCreedy et al. (1969) conducted bacterial acid leaching tests on a uranium ore using a semi-continuous, bench-scale, countercurrent-decantation system in which the ore was moved countercurrently to the flow of the leaching solution. Thiobacillus ferrooxidans provided the required oxidizing condition in the leach solution. The results indicated that 90% uranium extraction could be achieved from ores at pH 1.6 and 30°C. Acid consumption was equivalent to 25 lbs of H_2SO_4 per ton of ore compared to 60 to 80 lbs H_2SO_4 per ton obtained with conventional acid-leaching at 70°C.

BACTERIALLY ASSISTED PILOT PLANT PROCESS FOR LEACHING URANIUM ORES

Derry et al. (1977) developed a process for leaching uranium ore with bacterially regenerated lixiviant. An extraction efficiency of 94-95% from an ore containing 0.12% U_3O_8 was found after 10 days of leaching with a ferrous iron solution regenerated by Thiobacillus ferrooxidans. The researchers felt that the extraction rate could be raised as high as 97% with further refinement in the process.

Derry et al. (1977) feel that this process may have economic advantages over existing acid slurry leaching methods as a plant process. The bacterially generated leach solution had a small acid requirement in comparison to that of conventional acid leaching. A coarse particle size (-3 mm) was treated; this eliminated the need for fine grinding. Simple equipment was used in the leaching and bacterial liquor regeneration process.

FULL-SCALE URANIUM LEACHING AT AGNEW LAKE, ONTARIO

Agnew Lake Mines Ltd. applied in-situ leaching and surface heap leaching to Canadian uranium ore. The operation was designed to produce 1 million lb per year of U_3O_8 concentrate (Anon, 1978). Thiobacillus ferrooxidans was used to convert the uranium to the soluble hexavalent form. Complete technical details of the operation are given by Anon (1978).

Bacterial characterisitics of Agnew Lake Mine leach liquors were determined by Tuovinen et al. (1980b). Iron-oxidizing thiobacilli and glucose-oxidizing heterotrophs were detected in the leach liquor samples. Although the viable count of these organisms varied between <0.6 and 8.6×10^6 organisms/ml, the researchers found no evidence for seasonal fluctuations in bacterial numbers. There was a considerable variation in bacterial numbers at various sites in the mine but there were no consistently high populations at any one site. Filamentous fungi were detected in some leach samples but they were neither enumerated nor identified.

URANIUM LEACHING AT ELLIOT LAKE

A full-scale underground bacterial leaching program was conducted at the Milliken Mine of Rio Algom Mines Limited at Elliot Lake, Ontario in 1964 (Fisher, 1966). Over a one year period, 127,000 lbs of uranium were recovered using bacterial leaching. It was concluded that uranium could be recovered at a competetive cost with the utilization of bacterial leaching in a closed-down mine. The use of Thiobacillus ferrooxidans for the underground leaching required a minimum temperature of 18°C. During the winter the underground rock temperature dropped to 14°C and as a result, the bacterial activity decreased by 30 to 40% as compared to the summer.

Duncan and Bruynesteyn (1971) described the chemical oxidation and solubilization of uranium from Elliot Lake ores in the following reactions:

$$(1)\ UO_2 + Fe_2(SO_4)_3 + 2H_2SO_4 \rightarrow UO_2(SO_4)^{-4}{}_3 + 2FeSO_4- + 4H^+$$

$$(2)\ UO_3 + 3H_2SO_4 \rightarrow UO_2(SO_4)^{-4}{}_3 + H_2O + 4H^+$$

Ferrous iron can be oxidized back to the ferric state by Thiobacillus ferrooxidans:

$$(3)\ 2FeSO_4 + H_2SO_4 + \tfrac{1}{2}O_2 \rightarrow Fe_2(SO_4)_3 + H_2O$$

Ferric sulphate has limited solubility and hydrolyzes and precipitates as either ferric hydroxide (4) or basic ferric sulphate or jarosite-type mineral (5).

$$(4)\ Fe_2(SO_4)_3 + 6H_2O \rightarrow Fe(OH)_3 + 3H_2SO_4$$

$$(5)\ 3Fe_2(SO_4)_3 + 12H_2O \rightarrow 2HFe_3(SO_4)_2(OH)_6 + 5H_2SO_4$$

The bacteria can also attack pyrite to produce ferric iron and sulphuric acid:

$$(6)\ 2FeS_2 + 7\tfrac{1}{2}O_2 + H_2O \rightarrow Fe_2(SO_4)_3 + H_2SO_4$$

Duncan and Bruynesteyn (1971) found that there was a requirement for a nitrogen and phosphorous source during bacterial leaching but all other trace elements were supplied by the host rock.

Laboratory studies by Duncan and Bruynesteyn (1971) into the requirements of leaching bacteria indicated that the leaching area should contain an energy source; it should be damp not flooded; it should be within pH 2 or 3; there should be an adequate supply of oxygen and carbon dioxide; there should be a supply of ammonium sulphate and phosphate available for the bacteria.

BACFOX - A URANIUM LEACHING UNIT

The General Mining and Finance Corporation in South Africa installed a full-scale Bacfox unit at its Buffelsfontein plant in hopes that the uranium leaching rate would be raised by 4% through the bacterial oxidation of ferrous iron (Anon, 1977). In the Bacfox process, bacteria (Thiobacillus ferrooxidans) are immobilized in a reactor on a block of plastic resembling a honeycomb. Acidified ferrous sulphate is passed through the reactor and the bacteria oxidize ferrous iron to ferric iron. The ferric iron solution is used to leach uranium from an ore. During the leaching process, the ferric iron is reduced to the ferrous state. This ferrous iron can be re-oxidized to ferric iron by passing the leach solution through the reactor again. This biological regeneration of ferric iron, the key oxidizing agent in uranium leaching, was expected to replace a purely chemical extraction technique. Overall operating costs were expected to be cut by eight percent since the Bacfox operating costs are negligible.

MICROORGANISMS PLUG URANIUM OREBODIES

Accumulations of microorganisms in in situ leaching environments may plug orebodies and decrease uranium extraction (Brierley and Brierley, 1982). Microorganisms isolated at an in situ mining operation (Pseudomonads, Xanthomonads, Bacillus sp. and Micrococcus sp.) were inoculated into packed uranium ore columns to simulate leaching conditions. Permeability in two ore columns was reduced by one order of magnitude within 20 days. Hydrogen peroxide injected into the columns alleviated the microbial plugging problem; however, it is not known if the addition of hydrogen pyroxide will also kill Thiobacillus sp.

SECTION I.7

LEAD

LEAD

The principal lead mineral is galena (PbS) (Torma, 1978a). Lead ore deposits are usually associated with iron, zinc, copper, silver, gold, antimony, arsenic and bismuth impurities. Other lead minerals are oxides (PbO), carbonates (cerussite, $PbCo_3$) and sulphates (anglesite, $PbSO_4$). Few studies have been conducted on the bioleaching of lead.

MECHANISM OF GALENA OXIDATION

$$PbS + H_2SO_4 + \tfrac{1}{2}O_2 \rightarrow PbSO_4 + H_2O + S$$

Chemical oxidation of galena is incomplete due to the formation of lead sulphate and sulphur reaction layers covering the reactive sulphide surface (Tomizuka and Yagisawa, 1978). The role of bacteria is to oxidize sulphur to maintain the acid level and remove sulphur from the sulphide surface to allow further oxidation of sulphide to proceed.

OPTIMUM CONDITIONS FOR GALENA OXIDATION

Optimum conditions for galena oxidation in the presence of Thiobacillus ferrooxidans are:

1. galena particle size <50 microns (-300 mesh B.S. screen)
2. solids concentration lower than 4 g/100 ml of medium
3. pH 2.0

(Tomizuka and Yagisawa, 1978)

OPTIMUM LEACHING CONDITIONS FOR LEAD SULPHIDE CONCENTRATES

Torma (1978a) summarized the optimum microbial leaching conditions for off-grade lead sulphide concentrates in the following table:

TABLE I Optimum Conditions for Lead Sulphide Concentrates Leaching

Factors	Optimum Conditions
1.Temperature	35°C
2.pH	2.3
3.Eh	<500mV
4.Substrate concentrate (-400 mesh)	14-20% (W/V)
5.Bacterialinoculum	7%
6.Nutrients:$(NH_4)_2SO_4$	3g/l
K_2HPO_4	0.5g/l
CO_2 in air	0.2% (V/V)
O_2	an intensiveaeration is required
7.Substrate particle size	-325 to -400 mesh

SOLUBILIZATION OF LEAD SULPHIDES BY HETEROTROPHIC SOIL BACTERIA

Heterotrophic bacteria that solubilze lead sulphide (PbS) were isolated from soil (Cole, 1979). The bacteria capable of solubilizing PbS were identified by colony colour. The colonies were black or deep brown as a result of the precipitation of PbS in the colony. Two of the isolates produced metallic silver colonies on PbS agar. No attempts were made to fully identify the isolates. Isolates studied in detail were thought to belong to the genera, Pseudomonas or Xanthomonas. All isolates solubilized PbS but had little or no activity against $PbSO_4$, PbO or Pb_3O_4. Up to 0.356μg Pb^{2+} per ml of medium was solubilized during the active growth of the isolates; however, the amount solubilized depended on the particular isolate and medium composition.

The amount of lead directly associated with the cells was greater than that in solution.

When lead, zinc, cadmium, copper and iron sulphides occur together, it is virtually impossible to quantitatively recover the individual metals by conventional techniques due to the fine mineralization and differences in grindability (Torma, 1978a). Metal values associated with these intergrowths of sulphides may be recovered by microbiological leaching. During microbiological leaching, metal sulphides are oxidized to sulphates. Generally metal sulphates are soluble in aqueous solutions. Lead sulphate ($PbSO_4$) is an exception. This fact may be used to separate lead from solubilized metal values in the treatment of mixed metal sulphides.

A bacterial leaching process scheme for the recovery of copper, cadmium and zinc from a lead sulphide concentrate is outlined by Torma and Subramanian (1974). Thiobacillus ferrooxidans almost completely (<95%) removed the copper, zinc and cadmium values from the lead concentrate. The lead sulphide was partially converted to a sulphate during the oxidation process. Torma (1978a) suggests that a microbiological leaching process could exist in conjunction with an established pyrometallurigical lead smelting plant. The process produces a high grade lead concentrate (containing PbS or $PbSO_4$) which can be mixed with other concentrates to provide a feed for the smelter or sinter.

SECTION 1.8

ZINC

ZINC

Zinc easily forms a complex with sulphide to form the mineral sphalerite. Sphalerite contains 67% zinc (Zajic, 1969d). This mineral is usually associated with galena, pyrite, marcasite, chalcopyrite, smithsonite, calcite, and dolomite.

Zajic (1969d) feels that bipositive zinc found in sphalerite (ZnS), zincite (ZnO), and smithsonite ($ZnCO_3$) is not directly attacked by microorganisms. Zinc does not have many common valence states that would offer energy to an oxidizing microbe. Zinc sulphide, however, is readily oxidized to zinc sulphate which is soluble in aqueous solutions. The primary oxidation of zinc sulphides is most likely attributed to the ferric ions generated by the iron-oxidizing and sulphur-oxidizing bacteria.

The dissolution of zinc sulphide, ZnS, (eg. sphalerite) takes place by the following reaction (Zajic, 1969d):

$$ZnS + 2Fe_2(SO_4)_3 + 2H_2O + O_2 \rightarrow ZnSO_4 + 4FeSO_4 + 2H_2SO_4$$

delta $F_{25°C}$ = -122 kcal

Bacteria can oxidize pyrite to produce ferrous sulphate ($FeSO_4$). The ferrous sulphate is oxidized by the bacteria to yield ferric sulphate ($Fe_2(SO_4)_3$). Ferric sulphate is a strong oxidizing agent that converts the zinc sulphide (ZnS) to zinc sulphate ($ZnSO_4$) and ferrous sulphate. The zinc sulphate can be recovered and the ferrous sulphate is reoxidized by the bacteria to complete the cycle.

Theoretically, ZnS could undergo direct microbial oxidation by the following reaction:

$$ZnS + 2O_2 \longrightarrow ZnSO_4$$

This reaction is accomplished in metallurgical processes by roasting ZnS.

LEACHING OF ZINC SULPHIDE

A zinc sulphide was leached by Thiobacillus ferrooxidans in a continuous stirred tank reactor (Gormely et al., 1975). The zinc concentrate contained 55.7% zinc, 4.81% iron and 31.6% sulphur. The maximum rate of zinc release was 1.3 g/litre-hr. The maximum zinc extraction achieved was 71.9%. A model was developed to predict the leaching kinetics when bacterial growth rate was not limited by a substrate other than zinc concentrate.

MECHANISM OF ZINC SILICATE SOLUBILIZATION BY A MIXED POPULATION OF MICROORGANISMS

The solubility of willemite (Zn_2SiO_4) and synthetic willemite was studied in culture media innoculated with soil (Agbim and Doxtader, 1975). Up to 0.57% and 0.27% of the total zinc in the synthetic compound and the willemite were dissolved. The solubilization was directly related to pH. The concentration of zinc in solution was proportional to the hydrogen ion concentration. The researchers felt that chelation was not a significant mechanism by which zinc is solubilized in these experiments.

Groudev (1980b) observed that high rates of sphalerite oxidation were related to high levels of sulphur-oxidizing activity of Thiobacillus ferrooxidans strains.

LEACHING OF SPHALERITE CONCENTRATES

Dave et al. (1981b) studied the effect of pH, CO_2 availability, pulp density, particle size and amount of innoculum on the bacterial leaching rate of a sphalerite concentrate.

With a gradual increase in bacterial innoculum up to 11.0 mg dry weight, the zinc extraction increased. At higher doses of innoculum, the leaching rate decreased under the experimental conditions. The researchers felt that these results suggested that there is an optimum range for innoculum dosage. Any bacteria in excess of the optimum dosage would not find any free zinc surfaces for adsorption. An excess of cells that are metabolically inert may also have a depressing effect on the activity of others.

The pulp density (mass of solids/volume of medium x 100) had a significant effect on the leaching rate. The extraction rate increased directly with pulp density and reached its maximum value at 15-20% pulp density. The extraction rate decreased with pulp densities greater than 20%.

Enrichment of air with CO_2 increased both the leaching rate and zinc concentrates in solution. Without aeration, the total zinc in solution was 36.0 g/l. With air enriched with 0.1 to 2.0% CO_2, the zinc extraction rate increased and total zinc in solution increased to 70.5 g/l.

The optimum pH was 2.3. At 2.3, the lowest lag period, highest extraction rate, maximum percentage of leaching and maximum total zinc content were obtained. With pH >2.3, iron precipitated on the surface of the mineral particles and decreased activity. Maximum extraction with minimum iron precipitation corresponded with an optimum Eh value of 480-500 mV.

With -325 mesh particles, approximately 3 times more zinc was extracted compared to the amount extracted with -100+200 mesh particles. With the larger particles the extraction rate is limited by the small relative surface area of the particles.

EFFECT OF PARTICLE SIZE ON THE BIODEGRADATION OF A SPHALERITE CONCENTRATE

Torma and Guay (1976) studied the effect of particle size and total surface area on leaching using a sphalerite concentrate and an adapted strain of Thiobacillus ferrooxidans. The maximum zinc extraction rate was 574 mg/l/hr with a 10.1% substrate concentration. The final zinc yield was between 62 and 72% for the inoculated suspensions. Only 8 to 12% of the zinc was solubilized in the sterile controls. The extraction rate varied directly with substrate concentrations between 10 and 20%. At concentrations less than 10%, the extraction rate and bacterial growth were limited by substrate availability. At substrate concentrations greater than 20%, the oxygen mass transfer and carbon dioxide probably exerted limiting effects on the microorganisms.

The maximum zinc extraction rates were obtained with small particles that had a high surface area. However, Torma and Guay (1976) suggest that the optimum particle size used in large scale leaching operations will be dictated by the economics of the grinding costs and the gains which could be achieved by accelerating the leaching rate.

STATISTICAL OPTIMIZATION STUDIES ON LEACHING OF SPHALERITE

Dave et al. (1982) conducted statistical optimization studies on the bacterial leaching of sphalerite concentrates. The studies were confined to only one response, the percent extraction of zinc from sphalerite concentrates using Thiobacillus ferrooxidans. Factors affecting the percent zinc extraction were listed in decreasing order of their significance. These were particle size, pulp density, particle size x pulp density, pH, and cell mass. A mathematical model for the leaching process was developed in terms of the above parameters using an orthogonal composite design with three factors (pH, pulp density, innoculum concentration at a fixed particle size). The use of statistical design, analysis and optimization of a bioleaching process was also discussed.

ZINC-LEAD LEACHING

Ilyaletdinov et al. (1977b) suggested that bacterial leaching can be used for the selective extraction of metals from lead-zinc ores. The oxidation product of sphalerite, zinc sulphate, is dissolved in sulphate solutions containing Thiobacillus ferrooxidans cultures. Lead sulphate, the oxidation product of galemite, remains in the precipitate after the leaching. After zinc is removed from ore, the lead could be extracted with concentrated solutions of sodium chloride or with other solvents.

LEACHING OF A Zn-Pb-Cu ORE

Dutrizac (1979) subjected a pyritic zinc-lead-copper ore to percolation leaching using ferric sulphate-sulphuric acid solutions. Bacterial oxidation by Thiobacillus ferrooxidans was used to maintain a high Fe^{3+} concentration to investigate its effect on the extraction of zinc from the ore. Zinc was steadily leached from the 7.5 kg ore at 25°C. Approximately 50% extraction was achieved after 20 weeks. The final zinc concentration in 10 l of leaching solution was 25 g/l.

SECTION I.9

NICKEL

NICKEL

Pentlandite $(NiFe)_9S_8$ is the major nickel sulphide mineral. This mineral commonly occurs in ultrabasic rocks associated with pyrrhotite, chalcopyrite and cubanite (Dutrizac and MacDonald, 1974).

Little information has been published on the leaching of nickel sulphides. Corrans and Scholtz (1976) suggest that one reason for this fact may be that low ambient temperatures in countries such as Canada render bacterial dump-leaching of nickel impractical.

KINETIC MODEL FOR LEACHING OF NiS

Hendy and Rogers (1978) developed a kinetic model for the bacterial leaching of a synthetic nickel sulphide (NiS). The model was based on the following chemical and biological reactions:

1. Chemical leaching

$NiS + Fe(SO_4)_3 \longrightarrow NiSO_4 + 2FeSO_4 + S$

2. Natural oxidation

$NiS + 2O_2 \longrightarrow NiSO_4$ (pH=2.5) at low pH

3. Direct microbial action

$NiS + 2O_2 \xrightarrow{bacteria} NiSO_4$

4. Indirect microbial action

$4FeSO_4 + 2H_2SO_4 + O_2 \xrightarrow{bacteria} 2Fe_2(SO_4)_3 + 2H_2O$

LEACHING OF MILLERITE

Duncan et al. (1967a) leached 3.0 g of a 4% pulp of a nickel ore (millerite) using Thiobacillus ferrooxidans in a shake-flask at an initial pH of 2.5. After a six-day lag period, leaching was initiated. By the twentieth day, 100% of the nickel was solubilized. Only 25% of the nickel was solubilized by chemical leaching on the twentieth day. The maximum rate of nickel release with bacterial leaching was 6.3 mg per litre per hour. The researchers suggested that the rate of nickel release may be increased by using bacteria that have been pre-conditioned to high levels of nickel. The maximum concentration of soluble nickel that can be obtained with bacteria in a leaching solution was not established .

EFFECT OF pH ON NICKEL LEACHING FROM MILLERITE

Razzell and Trussell (1963) investigated the effect of acidity on the leaching of nickel from impure millerite. Bacterial leaching with Thiobacillus ferrooxidans was more rapid at pH 2.5 than at pH 4.0. After 5 days of leaching, 616 ppm Ni^{2+} was detected at pH 2.5; 231 ppm Ni^{2+} was solubilized at pH 4.0 during the same time period. Initially, bacterial leaching of the nickel from millerite was greater than spontaneous leaching but after 19 days of leaching there was no difference in the amount of nickel solubilized by bacterial and spontaneous leaching.

LEACHING OF PENTLANDITE

Dutrizac and MacDonald (1974) leached a crushed pentlandite ore in the presence and absence of Thiobacillus ferrooxidans by percolating acidified ferric sulphate solutions. Only minor differences were found in the nickel leaching rates with and without the bacteria being present. When bacteria were present, 0.71 g/l nickel and 0.62 g/l nickel were leached after 2015 and 2539 hours, respectively. Without bacteria, 0.26 g/l nickel was leached in 907 hours. The researchers suggested that the bacteria may have been inactive due to a lack of sufficient oxygen in the ore columns.

Torma (1972) studied nickel extractions on a naturally-occurring pentlandite-bearing sulphide ore and concentrate. The ore and concentrate contained 1.83% and 5.14% nickel, respectively. Both the ore and concentrate were ground by ball-mill to -400 mesh. They were leached by Thiobacillus ferrooxidans at pH 2.3 and 35°C. The air was enriched to 0.2% in CO_2 for aeration. As the nickel extraction progressed, iron precipitated in the form of basic sulphates. The surface of the sulphide materials were covered with a precipitate composed of ferric sulphate and gangue material when approximately 60% of the nickel had been extracted. As a result, bacterial activity was limited or inhibited. At the end of the experiments 63-82% of the nickel was extracted from the ore; 66-82% of the nickel was extracted from the concentrate. The extraction rate was found to increase linearly with an increase in pulp density up to about 18%. The maximum extraction rate was approximately 222 mg/l hr for the concentrate (25% pulp density) and 175 mg/l hr for the ore (25% pulp density). The rate increased faster with the concentrate than with the ore.

LEACHING OF COPPER-NICKEL DULUTH GABBRO ORE

Pentlandite, pyrrhotite and chalcopyrite/cubanite are the main sulphide minerals in Duluth gabbro. Both pyrrhotite and pentlandite are electrochemically in contact with less active chalcopyrite/cubanite; therefore, the galvanic dissolution of pyrrhotite and pentlandite from the gabbro ore and flotation tailings would enhance iron and nickel solubilization and acid generation (Natarajan and Iwasaki, 1983a).

The presence of bacteria would accelerate these galvanic interactions. Copper will dissolve in large concentrations only after most of the nickel has been solubilized.

Natarajan and Iwasaki (1983a) studied the leaching characteristics of copper-nickel bearing Duluth gabbro and flotation tailings in contact with water through agitation leaching in shake flasks (up to 8 months) and percolation leaching in columns (up to 96 months). The results of copper and nickel solubilization for the gabbro ore and flotation tailing in absence and presence of Thiobacillus ferrooxidans indicated that nickel release was faster and took place before copper was solubilized. More copper and nickel were released under acidic conditions than alkaline conditions. Although the bacterial leaching rates were highest under the acidic conditions, the organisms still survived at a high pH range (6-7).

The addition of calcite and mottled taconite tails to the ore or flotation tailings in shake flask experiments neutalized the acidity and therefore minimized heavy metal solubilization. After 120 days of leaching the flotation tailings and either calcite or mottled taconite tails, the amount of copper in the solution was negligible and the maximum amount of nickel extracted was 1.8 mg/l.

In the percolation leaching experiments on the gabbro ore, the copper and nickel concentrations in the solution after 24 months were 18 mg/l and 65 mg/l, respectively. After 72 months of leaching, the copper concentration exceeded that of the nickel concentration resulting in 825 mg/l copper and 530 mg/l nickel in the solution.

Additions of calcite and mottled tacenite tails also effectively neutralized acid production and decreased heavy metal ion release in the percolation leaching experiments. After a two year period the leachate from columns containing ore and 5% mottled tacconite tails contained 0.1 mg/l nickel and no copper.

BACTERIAL LEACHING OF NICKEL AND COPPER ORES

Golovko and Egorov (1974) studied the bacterial leaching of nickel and copper from a low grade-ore of the Kola Peninsula, U.S.S.R. Thiobacillus ferrooxidans leached 74.1% of the original nickel content and 22.5% of the copper content in 530 days. In the control, 29.1% of the nickel and 11.9% of the copper was extracted.

The dynamics of the leaching of the nickel was divided into three periods which had different rates of nickel transfer into the solution:

1. First 250 days - the period of intensive leaching.
2. From 300-400 days - steady leaching.
3. From 400-500 days - slow leaching.

The highest consumption of sulphuric acid was in the first 100 days of leaching.

BATCH MICROBIOLOGICAL METAL EXTRACTION

Based on his experimental work on nickel and cobalt extractions, Torma (1972) suggested a process scheme for batch microbial metal extraction. The two-step biohydrometallurgical process extracts up 50 to 60% of the metal values in the first step. The solid ore is then reground and releached to complete the second step. Metals are recovered after the filtrate is purified and the iron is precipitated. The leaching solution is recycled after its pH and nutrient concentrations have been adjusted.

SECTION I.10

BAUXITE

BAUXITE

Bauxite is the primary source of aluminum. It is a mixture of hydrous aluminum oxides of indefinite composition (Zajic, 1969a). Most bauxites are usually composed of gibbsite ($Al(OH)_3$), boehmite ($AlO(OH)$), diaspore ($HAlO_2$) and iron. Cliachite ($Al(OH)_3$) is the amorphous constituent in bauxite.

The basic impurity in bauxite is silica which occurs in the make-up of different aluminosilicates. High silica bauxites are characterized by the exceptionally fine, almost emulsion-like dispersion of minerals occurring with alumina in a cemented mass of rock. The minerals are tightly intergrown. Low grade, high silica bauxite cannot be processed into alumina by the Bayer process unless they are enriched (Andreev et al., 1976). In conventional enrichment processes, SiO_2 is removed but large amounts of Al_2O_3 are also lost (Andreev et al., 1976). Effective enrichment of high silica bauxites can also be complex and expensive. Andreev et al. (1976) proposed that one of the most promising methods of bauxite desiliconization is by the leaching of aluminosilicates with microorganisms.

Andreev and Pol'kin (1981) found that microbial leaching was effective for various types of bauxites and produced high-grade aluminum concentrates. Complex aluminosilicates (e.g. chamosite) were leached most strongly. Kaolinite was not leached until after the more complex aluminosilicates hade been decomposed to a large degree. In bauxites with a high content of kaolinite (allites), kaolinite decomposition was initiated at the same time as the decomposition of chamosite but kaolinite decomposition occurs much more slowly. During the leaching of kaolinite bauxites and allites, aluminum oxide was released into the bacterial solution. A small part of the aluminum oxide formed synthetic gibbsite. As the leaching time was increased, the amount of gibbsite formed increased. During the leaching of chamosite-containing ores, the aluminum oxide formed gibbsite and kaolinite. However, as the leaching time was increased, the amount of aluminum oxide going into solution increased. Bacterial leaching for the desiliconizing of bauxites was highly selective and up to 100% extraction of aluminum oxides could be achieved.

MICROORGANISMS INVOLVED IN THE LEACHING OF ALUMINOSILICATES

BACILLUS MUCILARGINOSUS

Andreev et al. (1979) described the Bacillus mucilarginosus bacteria as gram-negative rods covered by a capsule. Spores produced by the bacteria are oval. On a nitrogen-free medium, these bacteria form round, complex, transparent colonies with rounded edges. Under favorable conditions these bacteria break down silicates and aluminum silicates to release silicon (Andreev et al., 1979). As a result, high-grade concentrates are produced from high-silica, low-grade bauxites.

EFFECT OF METAL CATIONS ON BACILLUS MUCILARGINOSUS

During the bacterial leaching of bauxites, metals such as aluminum, silicon, iron, and calcium are solubilized. Andreev et al. (1982) studied the effect of these metals on the growth and multiplication of silicate bacteria.

At low concentrations (10 mg/l), aluminum and its hydroxide stimulated multiplication of the cells. Higher concentrations of aluminum had an adverse effect on the bacteria.

Concentrations of the salts of trivalent iron up to 40 mg/l also stimulated growth. The bacterial concentrations increased to 10^7 cells/cm^3. Concentrations of iron above 40 mg/l decreased bacterial populations but 10^5 cells were still present at 50 g/l iron. Divalent iron and sodium silicate cations had no effect on the activity of the bacteria. Calcium chloride had a slightly inhibitory effect.

Bacterial suspensions of a three-day-old culture were analyzed and significant amounts of amino and organic acids were found. The researchers believe that these amino and organic acids complex with the metal cations to prevent the unfavorable effects of metal cations on growth and multiplication of silicate bacteria.

EFFECT OF THE COMPOSITION OF THE CULTURE MEDIUM ON THE BACTERIAL DECOMPOSITION OF ALUMINOSILICATES.

Bacteria actively leached silica from an ore regardless of the composition of the culture medium; however, the most significant leaching occured when the culture medium contained carbohydrates (glucose) at a concentration of 1% (Andreev et al., 1979). Approximately 60% of the silica was solubilized and 68% of the aluminum was extracted into the concentrate. The quality of the concentrate obtained was improved by 1.68 times compared to the initial ore. In a mineral culture medium, 72.2% of the aluminum was extracted into the concentrate but only 45% of the silica was removed from the original ore. The quality of the obtained concentrate was only improved by 1.37 times.

Continuous replacement of the bacterial solutions to remove metabolites and leaching products resulted in more extensive desiliconizing of ore. In a carbohydrate medium, the bacterial solution was changed twice. The quality of the obtained concentrate was 2.4 times higher compared with the initial ore. Approximately 71% of the aluminum was extracted into the concentrate. More than 65% of the silica was leached from the ore.

HETEROTROPHIC BACTERIA

Heterotrophic bacteria were used to desiliconize two high silicide, low grade bauxites (Andreev et al., 1976). The first bauxite ore was either treated with bacteria for seven days or enriched by flotation. With bacterial enrichment, the Al_2O_3 content increased from 42 to 53.6%. Approximately 88.2% of the Al_2O_3 was recovered. The SiO_2 content was decreased from 27.7 to 18.3%. The remaining silica was in the form of quartz and the researchers did not consider it a harmful impurity in the production of Al_3O_2 by the Bayer method. Enrichment by flotation only increased the Al_2O_3 content to 48.4%; the SiO_2 content was lowered to 21.1%. The other bauxite ore was also treated with heterotrophic bacteria for seven days but was subsequently washed with hot water twice. The Al_2O_3 content was increased from 47.9 to 58.7% with a recovery of 87.2%. The SiO_2 content decreased from 12.6 to 5.8% and the grade of the bauxite was improved enough to be successfully converted to alumina by the Bayer method. Other bauxite samples were enriched by magnetic separation and a concentrate was obtained that contained 53.2% Al_2O_3 and 10.1% SiO_2. Only 65.3% Al_2O_3 was extracted. This bauxite concentrate required both the Bayer method and sintering for its successful conversion to alumina.

EFFECT OF BACTERIA ON SILICA OF BAUXITE ORES

Contradictory to the reports from the Soviet scientists, Silver (1983) found that Aspergillus niger, Pseudomonas ATCC 21025 and Bacillus mucilaginosus did not solubilize reactive silica from bauxite ores and kaolin preparations sufficiently for commercial exploitation. These microorganisms extracted silicon from kaolin and kaolin clay to yield concentrations of only 0.008 to 0.030 g/l. This maximum yield of 0.03 g/l was only six times the solubility concentration of silicon (0.005 g/l) and Silver (1983) concluded that this was insufficient for commercial exploitation.

Arthrobacter globiformis, A. simplex, A. tumescens, Nocardi farcinica, Azotobacter beijerinckii and eight unidentified species of bacteria from the genera Arthrobacter and Nocardia were isolated from various bauxite ores. None of these bacteria could release silicon from different bauxite ores or kaolin clays into solution to a concentration greater than 0.044 g/l.

Carboxilic acids produced by microorganisms could not extract silicon from kaolin clay in sufficient quantities for commercial exploitation (Silver, 1983). Citrate, itaconate, 2-ketogluconate, malate or tartarate extracted silicon from the clay to yield concentrations of silicon in solution between 0.004 and 0.014 mg/l. Since the concentration of the solubility of aluminum silicates in water is 0.005 g/l, Silver (1983) concluded that carboxylic acids alone were not capable of extracting silicon from kaolinite.

SECTION 1.11

LEUCITE

LEACHING ALUMINUM AND POTASSIUM FROM LEUCITE

Leucite is a potassium aluminum silicate ($((2SiO_2AlO_2)^{-1}K^{+1})$ (Rossi, 1978). It generally contains 21.58% K_2O and 23.4% Al_2O_3. The success of bacterial leaching on sulphide minerals has prompted investigations into the feasibility of solubilization of aluminum and potassium from leucite.

Rossi (1978) studied the solubilization of potassium and aluminum from leucite using heterotrophic bacteria and thiobacilli in batch cultures. The leucite used in the experiments was a concentrate obtained from volcanic rock in the Civitacastellana area of Italy. It contained 55.44% SiO_2 and 22.58% Al_2O_3. *Thiobacillus ferrooxidans* was used for leaching experiments with mixtures of leaucite and pyrite. For leaching studies on leucite, heterotrophic microorganisms isolated from Italian soil or from collections were used. The microorganisms obtained from collections were *Scopulariopsis brevicaule*, *Penicillium expansum* Link and *Aspergillus niger* van Tieghen.

Thiobacillus ferrooxidans was not capable of leaching leucite in the presence of pyrite. After one month, the bacteria seemed to be inhibited. Rossi (1978) concluded that an unknown soluble component of the leucite mineral was toxic to the bacteria.

Leaching experiments conducted with microorganisms isolated from leucite soils also gave negative results. The leucite, however, was not toxic to soil microorganisms. Other strains of the isolated microorganisms may demonstrate a greater ability to solubilize potassium.

The strains of Scopulariopsis brevicaule and Penicillium expansum leached 27% and 22% of the potassium from the leucite, respectively. The potassium solubilization rate continually decreased as the experiments proceded. Rossi (1978) suggested that this may be due to the formation of a protective coating on the surfaces of the mineral particles which effectively inhibits an indirect chemical attack on the leucite.

SECTION I.12

GOLD

GOLD

PRE-OXIDATION OF GOLD CONCENTRATES BY BACTERIAL LEACHING

Lawrence and Bruynesteyn (1983) investigated the pre-oxidation of refractory pyrite gold concentrates by bacterial leaching to enhance the recovery of gold and silver in cyanidation. For one concentrate, a gold recovery of 81% was achieved following an 87% pyrite oxidation. Direct cyanidation of this concentrate resulted in 24% gold recovery. Gold and silver recoveries from other concentrates increased from 60-78% to more than 90% and 80-86% to more than 98%, respectively, following biological pyrite oxidation to 80-90%.

GOLD DISSOLUTION BY BACTERIA

Korobushkina et al. (1983) arranged microorganisms in order of ability to concentrate gold: actinomycetes > fungi > bacteria. The most active microorganisms in the dissolution of gold were Bacillus megaterium, Bacillus mesentericus and Pseudomonas liquefaciens. The dissolving power of these bacteria increased by selection of the nutrient media and by adding sodium peroxide to the solution as an oxidant for gold (Korobushkina et al., 1974).

Upon further investigations, Korobushkina et al. (1976) found that amino groups were involved in the formation of gold-amino acid complexes; functional groups of amino acid residues in polypeptide groups participated in the formation of gold-protein complexes. The bonding of gold to amino acids and proteins was thought to be accomplished through the nitrogen of the amino group. Amionic-gold complexes were most prevalent.

Nucleic, aspartic, glutamic, pyruvic, lactic, formic and acetic acids as well as peroxidase, catalase and proteolytic enzymes were found in culture media of bacteria from gold deposits (Korobushkina et al., 1983). Gold was dissolved by amino acids in the presence of Na_2O_2 and under alkaline conditions. In 30 days, 0.03, 0.04 and 0.11 mg Au/l were dissolved in the presence of asparagine, aspartic acid and threonine, respectively. At low pH values, 0.01-0.1 mg Au/l dissolved in the same time period.

Dissolution of gold dust in the presence of an oxidant (Na_2O_2) was most active in the presence of histidine, serine, aspartic acid, glycine, alanine, methionine and asparagine. Gold was also dissolved in the presence of nucleic acids by peroxide. Salt-and alkaline-soluble proteins and water-and alcohol-soluble proteins were less effective in dissolving gold.

Korobushknia et al. (1983) claim that gold (III)-amino acid interactions at pH 2 results in a slow reduction of gold to the metallic state whereas gold salts form in alkaline conditions. With large quantities of amino acids present, gold (III) is reduced to gold (I). The stability of gold (I)-amino acid complexes varies with their redox potential.

The complex-forming capacity of amino acids was ranked according to the redox potentials as follows:

cysteine > histidine > asparagine > methionine > glycine, alanine, phenylalanine.

CHANGES IN GOLD DURING BACTERIAL DISSOLUTION

Korobushkina et al. (1977) studied the influence of the activity of accumulating and pure bacterial cultures, as well as amino acid and protein fractions isolated from the strain Bacillus mesentericum niger 12 on powdered and placer gold with and without the addition of sodium peroxide. Pseudomonas fluorescens, Sarcina alba, Bacterium nitrificans, Bacillus mesentericus niger, and Bacillus oligonitrophilus dominated in the accumulating culture. It was shown that with increasing the leaching time, there was an accumulation of metal in the solvent.

The maximum gold content in the solution after 360 days of leaching (64.4 mg/l) was obtained by the dissolution of powdered gold by the protein fraction isolated from the culture fluid of Bacillus mesentericus niger. Only 7.5 mg/l of gold were obtained through the activity of the culture fluid of the accumulating cultures on placer gold after 1080 days.

Qualitative changes in the gold were only detected after 30-day contact with the bacterial solvents and were expressed by changes in the colour of the gold. After 150-day experiments, the gold acquired rough and etched surfaces and lost its strong metallic luster. In experiments where the placer gold was in contact with the culture fluid of accumulating cultures for three years, the particle size of the gold was reduced by 1.5-2.0 fold.

Micro-x-ray analysis indicated that silver was removed from the gold during the leaching process. Before leaching, the gold pieces contained 82.1% gold and 17.6% silver. After leaching, the amount of silver was reduced to 1%. The removal of silver resulted in an increase in the strength of the gold in the edges of the gold pieces.

SECTION I.13

ARSENIC

ARSENIC

Metal sulphide concentrates are usually contaminated with gangue materials which are often difficult to remove during the concentrating and refining stages. Arsenic, which is frequently found in the form arsenopyrite, is a common contaminant. Although arsenic can be removed by volatilization, the arsenic trioxide (gas) recovered is highly toxic. Considerable expense is incurred in the production and safe removal of this gas (Pinches, 1975). Bacterial leaching is an alternative treatment for the removal of arsenic.

BACTERIAL LEACHING OF ARSENOPYRITE

Pinches (1975) outlined the following chemical reactions that are thought to occur in the bacterial leaching of arsenopyrite. Arsenopyrite (FeAsS) is oxidized by *Thiobacillus ferrooxidans*. This reaction produces arsenic acid (H_3AsO_4) and ferrous ($FeSO_4$) and ferric ($Fe_2(SO_4)_3$) sulphate. Arsenous acid may be produced in an intermediate stage.

$$(1)\ 2FeAsS + 6\tfrac{1}{2}O_2 + 3H_2O \xrightarrow{\text{bacteria}} 2H_3AsO_4 + 2FeSO_4$$

$$(2)\ 2FeSO_4 + H_2SO_4 + \tfrac{1}{2}O_2 \xrightarrow{\text{bacteria}} Fe_2(SO_4)_3 + H_2O$$

The arsenopyrite may also be oxidized by the ferric sulphate formed. This reaction produces ferrous sulphate which can be reoxidized by the bacteria by equation (2).

$$(3)\ 2FeAsS + Fe_2(SO_4)_3 + 4H_2O + 6O_2 \rightarrow 2H_3AsO_4 + 4FeSO_4 + H_2SO_4$$

Ferric sulphate can also react with arsenic acid which precipitates as iron arsenate ($FeAsO_4$).

$$(4)\ Fe_2(SO_4)_3 + 2H_3AsO_4 \longleftrightarrow 2FeAsO_4 + 3H_2SO_4$$

At higher pH values (>pH 2), the iron arsenate can be hydrolyzed.

$$(5)\ FeAsO_4 + 3H_2O \longleftrightarrow Fe(OH)_3 + H_3AsO_4$$

At pH 2 or higher, ferric ions will precipitate due to the hydrolysis of ferric sulphate.

$$(6)\ Fe_2(SO_4)_3 + 6H_2O \longleftrightarrow 2Fe(OH)_3 + 3H_2SO_4$$

Temperature, solution pH, mineral surface area and availability of inorganic nutrients can affect the leaching of a pyrite-arsenopyrite concentrate by Thiobacillus ferrooxidans (Pinches, 1975).

The optimum temperature for arsenic extraction was between 32-35°C. Metal extraction rates rapidly declined if the temperatures were above the optimum range.

The bacterial leaching of 1.5% (w/v) pyrite-arsenopyrite could only be initiated in the pH range of 1.5 to 4.4. The initial pH significantly affected the amount of leach products precipitated. As initial pH values increased, precipitation also increased. The leach products were precipitated due to the formation of iron arsenate and the hydrolysis of ferric sulphate and iron arsenate. This hydrolysis acted as a buffer to the system. At lower initial pH values, the acidic ferric sulphate generated from the leaching of pyrite-arsenopyrite particles depressed these reactions and reduced the precipitation of the leached products.

Surface area was an important factor in determining the leaching rate of the arsenopyrite particles in the sulphide concentrate. As surface area increased, the rate of leaching became fairly constant. Pulp density also affected the arsenic leaching rates. At low pulp densities, the leach rate was directly proportional to the pulp density but it became more independent as the pulp density was increased. Pulp density also affected the amount of arsenic leached. At 1.5 - 2.0% pulp density, 100% of the arsenic was leached. As pulp density increased, the amount of leached arsenic decreased. For example, at 20% pulp density, only 54% of the arsenic was leached.

Bacterial growth studies were conducted as batch tests (using 1.5% (w/v) pulp densities of pyrite-arsenopyrite concentrate in the mineral salts medium) to determine the relationshiip between bacterial growth and leaching. During the growth and leaching phases, the bacterial cells were associated with the mineral particles. Only actively growing cells were associated with the mineral surfaces. A pale-green surficial layer of iron, arsenic and sulphur was formed around the arsenopyrite particles being leached. This layer increased as the leaching process progressed. This surficial coating was found to be soluble in hydrochloric acid.

OXIDATION OF ARSENOPYRITE AND ARGENITE BY THIOBACILLI

Ehrlich (1964) found that members of the Thiobacillus group of bacteria accelerated the oxidation of arsenopyrite during 21 days of incubation. As a result of this oxidation, arsenic was solubilized as arsenite and arsenate. The iron present in the arsenopyrite was oxidized and precipitated as iron arsenite and arsenate.

During the bacterial oxidation of enargite, approximately six times as much copper and arsenic were brought into solution by bacteria as compared to that brought into solution by spontaneous, chemical oxidation during 21 days of incubation. The copper and arsenic were solubilized in the cupric and arsenate forms.

OXIDATION OF ARSENOPYRITE BY PURE AND MIXED CULTURES OF THIOBACILLUS FERROOXIDANS AND THIOBACILLUS THIOOXIDANS

Groudev (1981) studied the oxidation of arsenopyrite by pure and mixed cultures of Thiobacillus ferrooxidans and Thiobacillus thiooxidans. The bacteria were adapted to grow in the presence of 2.5 g/l dissolved arsenic. Pure cultures of Thiobacillus thiooxidans did not oxidize any arsenopyrite; pure cultures of Thiobacillus ferrooxidans were able to leach 68% to 74% of the arsenic from an arsenopyrite containing 43.77% arsenic. The addition of ferrous iron accelerated the arsenic dissolution rates by Thiobacillus ferrooxidans. Groudev (1981) concluded that this finding indicated that the indirect Fe^{3+}-dependent oxidative mechanism played a significant role in the oxidation of arsenopyrite. The addition of Thiobacillus thiooxidans to cultures of Thiobacillus ferrooxidans had no effect on the arsenopyrite oxidation rates or the amount of arsenic solubilized.

OXIDATION OF AS(III) TO AS(V) BY PSEUDOMONAS ARSENITOXIDANS

Ilyaletdinov and Abdrashitava (1981) isolated a microorganism from the mine waters of a gold-arsenic deposit that oxidizes trivalent arsenic to the pentavalent form under autotrophic conditions. The isolate, Pseudomonas arsenitoxidans, is capable of growing and receiving energy from the oxidation of trivalent arsenic compounds.

Ilyaletdinov and Abdrashitava (1981) describe Pseudomonas arsenitoxidans as aerobic, gram-negative, nonspore-forming rods, 0.4 x 1.2 - 1.5 µm in size. They occur as motile, single cells possessing one flagellum. The cells are capsulated. The optimum pH for growth and activity is between 6.0 and 8.5. The organism does not utilize alcohols, carbohydrates or organic acids as energy sources. It is capable of oxidizing trivalent arsenic to the pentavalent form. A culture can oxidize 1000 mg/litre arsenic in 20 days. The addition of 0.05 mg/l yeast autolyzate increases the oxidation rate. The NH_4^+ ion is required as an energy source.

OXIDATION OF ARSENIC BY PSEUDOMONAS PUTIDA AND ALCALIGENES EUTROPHUS

Abdrashitova et al. (1981) isolated two heterotrophic bacteria, Pseudomonas putida and Alcaligenese eutrophus, from gold-arsenic deposits which were capable of oxidizing As(III) to As(V) at pH 6-9 and temperature 4-28°C and 28°C, respectively. The oxidation of arsenic was accompanied by a decrease in pH to 4-5. The rate of oxidation of As^{3+} was dependent on the number of cells in the inoculum.

VOLATILE AND SOLUBLE ARSENIC RELEASE FROM SHALE

High arsenic levels are found in retorted shales. Klein and Hassler (1981) studied the effect of microbial growth on volatile and soluble arsenic release from soil-retorted shale mixtures. The retorted shale was able to serve as a source of nitrogen for bacterial growth but was an extremely poor source of carbon. Microbial population levels increased with glucose and ammonium additions to the shale. The highest arsenic levels, 0.47 µg/ml and 0.64 µg/ml, were found in the control and nitrogen-ammended shale slurries, respectively. These conditions also resulted in the least amount of bacterial and fungal growth. The highest viable populatons were found in glucose and glucose-NH_4NO_3 amended slurries but the lowest supernatant arsenic levels (0.1-0.44 µg/ml) were found in these slurries. Klein and Hassler (1981) concluded that arsenic may have been immobilized by increases of bacterial and fungal biomass, or lost by increased volatilization.

The pH values of the retorted shale slurries increased after glucose ammendments. A relationship between pH changes and supernatant arsenic levels was not established.

Twelve-week experiments investigated the role of microorganisms in arsenic volatilization from retorted shales. The addition of dimethylarsenic acid (DMA) and nutrient amendments increased arsenic volatilization, which was highest under aerobic conditions and in the presence of retorted shale, in comparison with added soil.

LEACHING OF AN ARSENIC-TIN-COPPER CONCENTRATE

A concentrate containing 2.31% tin, 8.77% copper, and 6.5% arsenic was bacterially leached at 28-30°C (Pol'kin et al., 1975). When the arsenic was leached in counter-current, 91.5% of the arsenic was extracted in 100 hrs. as compared to 39% in a direct current leaching experiment.

SECTION I.14

OIL SHALE

BIOLEACHING OF OIL SHALE

Oil shale is a fine-grained sedimentary rock containing between 5% and 65% kerogen. Upon destructive distillation-retorting, the kerogen yields oil (Chiang et al., 1981). This retorting method is energy-consuming and requires elevated temperatures to break the kerogen into oil. The high temperatures cause organic chemicals to form that are difficult to refine into fuel.

Chiang et al. (1981) subjected a crushed oil shale to leaching by stationary, shaking and continuous cultures of Thiobacillus thiooxidans (ATCC 8085).

In the stationary culture, the pH dropped as a function of time due to the production of sulphuric acid by the activity of Thiobacillus thiooxidans. The floating sulphur precipitated due to the presence of extracellular lipids which are produced by the bacteria and serve as a wetting agent.

Thiobacillus thiooxidans is an aerobe and thus the effect of stirring and aeration on the activity of the bacteria was investigated. The microorganism exhibited a four to five-fold increase in the rate of production of sulphur acid as compared with that of a stationary culture.

During the initial stage of acid leaching, the reaction rate depended on the availability of surface area of the sample. At later stages of leaching the reaction was slowed down due to the pore diffusional limitations. According to theory, the maximum removable mineral in the rushed shales of a specified particle size was approximately 25% of the original shale weight. The leaching rate was not controlled by acid concentrations of 0.1 - 1.0 N but depended on the amount of shales leached per equivalent of acid applied. Over 90% of the carbonate minerals were removed from shale matrix in 14 days of exposure to 20 g. shale per acid equivalent.

Chiang et al. (1981) stated that the bioleaching of shale is an economical method of shale oil recovery.

PURIFYING OIL SHALE RETORT WATER

Oil shale retort water contains considerable quantities of soluble organic and inorganic materials. Yen and Findley (1978) patented a process for purifying oil shale retort water containing a substantial amount of organic and inorganic components (including sulphate) to enable the growth of bacteria of the Desulfovibrio family. The members of the Desulfovibrio family that can be used for this purpose are Desulfovibrio desulphuricans (especially the subspecies aestuarie and desulphuricans), Desulfovibrio africanus, Desulfovibrio gigas, Desulfovibrio salexigens, Desulfovibrio vulgaris (particularly subspecies oxamicus and vulgaris). These bacteria required neutral or only slightly alkaline or acidic water for growth, therefore, the pH of the water should be maintained in the range 6.5-7.5. These anaerobic bacteria utilize the organic, as well as the ammonia, phosphate and sulphate components, for growth. The sulphate is used as an electron accepter in the Desulfovibrio's metabolic processes and is converted to sulphide. As the cell biomass grows, it forms a flocculent mass which can be removed by skimming from the vessel. The sulphide ions in the water are then oxidized to sulphate. This may be achieved by adding aerobic bacteria to the retort water and aerating. The oxidation prevents further growth of the anaerobic bacteria. Members of the aerobic Thiobacillus family which may be used are Thiobacillus thiooxidans, Thiobacillus concretivorous, Thiobacillus sulfooxidans, Thiobacillus ferrooxidans, Thiobacillus thioparus, Thiobacillus intermedius, Thiobacillus novelius, Thiobacillus *thermitanus*, *Thiobacillus umbonatus*, *Thiobacillus lobatus*, *Thiobacillus crenatus* and *Thiobacillus neopolitans*.

A portion of the sulphate-containing retort water can be recycled back to neutralize new retort water. The remaining sulphate is neutralized by reaction with the inorganic matrix (carbonate, quartz and silicate minerals) of retorted oil shale. Thiobacillus species do not flocculate, therefore, the dead Thiobacillus cells agglomerate with the reacted spent shale. The slurry of shale, dead Thiobacillus cells and retort water is allowed to settle in a settling tank and the overflow can be removed to be used as industrial grade water for the mining and retorting operation. The spent shale can be combined with the flocculated Desulfovibrio biomass and used as fertilizer. The cell biomass can also be recycled to the retort water as a nutrient source for bacteria.

SECTION I.15

VANADIUM

VANADIUM

Vanadium is used as an additive in high-strength low-alloy steels. The largest deposit of vanadium in Canada is in the bituminous Athabasca oil sands in Alberta (Sullivan et al., 1980b) and approximately 24 Tg of vanadium is present in the bitumin available for surface mining. Vanadium is concentrated into petroleum cokes during the upgrading of bitumen for oil recovery. The amount concentrated ranges from 800-8000 µg/g and depends on the coking process employed.

Two types of coke, viz. delayed coker (produced by Great Canadian Oil Sands Ltd.) and fluid coker (Syncrude Canada Ltd.) are not amenable to leaching (Sullivan et al., 1980b). Sullivan et al. (1980b) reported various leaching characteristics of an experimental fluid coke (produced by Exxon).

Thiobacillus thiooxidans ATCC 8085 provided the acidic conditions for leaching. An elemental sulphur supplement was added to the coke since the bacteria were not able to use the sulphur content of the coke (1.6%). In leaching tests conducted in airlift percolator columns, approximately 30% of the total vanadium content was extracted during microbial leaching at pH 1. This was similar to the extraction achieved by chemical leaching at the same pH.

During the leaching of flexicoker coke derived from Athabasca oil sands bitumen, the following metals are released (Jack et al., 1980): Cr, Co, Cu, Fe, Mn, Mo, Ni, Sr, V, Zn. Vanadium is usually present in the highest concentrations. In acidic conditions, vanadium can exist as either vanadium (IV) or vanadium (V).

The growth of Thiobacillus thiooxidans ATCC 8085 was inhibited by vanadium (V), molybdenum (VI) and chromium (VI) (Jack et al., 1980).

Vanadium (IV) did not prevent the growth of the bacteria when it was present in the medium as 2.7×10^{-2} - 5.6×10^{-2} M vanadyl sulphate. Vanadium (V) prevented the growth of the bacteria when added to the medium as sodium ortho-vanadate before inoculation or during a well established exponential growth phase. Viable cells were able to reduce vanadium (V) to the non-toxic vanadyl ion. The kinetics of this reduction reaction were first-order in vanadium (V) concentration with a half-life of 10 hours. Concentrations of vanadium (V) above the reducing capacity of the culture prevented further microbial growth. The actual toxicity of vanadium depended on the reducing capacity of the culture.

Ni^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+}, and Sr^{2+} had no significant effect on the growth of Thiobacillus thiooxidans. Molybdate and chromate, however, inhibited growth when present in concentrations of 2 to 5×10^{-4} M.

The actual mechanism of vanadium (V), molybdenum (VI) and chromium (V) toxicity is not known. In any leaching application that uses Thiobacillus thiooxidans, however, the release of these metals into the leaching solution may limit microbial growth and metal recovery.

SECTION I.16

FOSSIL FUEL

OIL INDUSTRY

Currently, enhanced oil recovery techniques involve the injection of heat or chemicals into an oil well to lower the viscosity of the oil to enhance the recovery from a reservoir (Anon, 1982). Microorganisms could produce the chemicals (surfactants polymers, solvents, gases etc) which are injected into the well or alternatively, the microorganisms themselves could be injected into the well to lower the oil viscosity.

Research and development of this particular application of biotechnology was delineated in the following areas:

1. high pressure/temperature/salinity tolerance
2. ability to use oil substrates
3. increased production of gases and chemicals to reduce oil viscosity.

IN SITU MINING OF FOSSIL FUEL

Ghosh and Klass (1978) patented a process for recovering underground fossil fuel deposits retained in an organic matrix by using anaerobic microorganisms. The microorganisms produce particles of the deposit of a size that form a slurry with an aqueous medium that can be pumped to the surface where further processing can be conducted for the recovery of the desired organic hydrocarbon fraction. Kerogen from oil shale and bitumens from tar sands can be recovered by this method. The anaerobic microorganisms may produce gaseous hydrocarbons during contact with the underground fuel deposit. These hydrocarbons are suitable for use as fuels in the surface treatment processes for the separation of the organic carbonaceous component from the inorganic material that require heat.

The anaerobic microorganisms use the inorganic matrix of the underground formation as a nutrient. Suitable anaerobes include Clostridium, Pseudomonas, Esherichia Coli, Methanobacterium, Methanosarcina bakerii, Methanococcus vannielle or mixed cultures of the above mentioned bacteria.

SECTION 1.17

COAL

COAL

Coal with high levels of sulphur cannot be used because upon direct combustion the sulphur is released into the atmosphere mainly as the pollutant, sulphur dioxide. Desulphurization of coal can be achieved by bacterial leaching.

Up to 70% of the sulphur contained in coal can be in the form of pyritic sulphur (Kelly et al., 1979). The pyritic sulphur commonly occurs as iron pyrite or marcasite. Both of these minerals have the same chemical composition (FeS_2) but have different crystalline structures (Murr and Mehta, 1982).

FeS_2 can be oxidized to soluble sulphate by the following reactions:

$$(1)\ FeS_2 + Fe_2(SO_4)_3 \longrightarrow 3FeSO_4 + 2S$$
$$(2)\ FeS_2 + 3.5O_2 + H_2O \longrightarrow FeSO_4 + H_2SO_4$$
$$(3)\ FeS_2 + 3\ O_2 + 2H_2O \longrightarrow 2H_2SO_4 + Fe^{3+}$$

Reaction (1) is the basis for chemical coal desulphurization by ferric sulphate leaching. The reaction can be rapid at high temperatures (greater than 100°C) and high ferric sulphate concentrations ($Fe_2(SO_4)_3$) but these conditions can create major corrosion problems in equipment (Murr and Mehta, 1982). The elemental sulphur produced by the reaction must be removed.

Reactions (2) and (3) are slow but in the presence of bacteria, reaction (2) is accelerated. In addition, bacteria can convert a product of reaction (1), ferrous sulphate ($FeSO_4$) to ferric sulphate ($Fe_2(SO_4)_3$) by the following reaction

$$(4)\ FeSO_4 + 0.5\ O_2 \longrightarrow Fe_2(SO_4)_3 + H_2O$$

Ferric sulphate can then react with the pyrite by reaction (3). Any elemental sulphur produced is converted by the bacteria to sulphuric acid.

$$(5)\ 2S + 2H_2O + 3O_2 \longrightarrow 2H_2SO_4$$

Microbial coal desulphurization (MCD) has no severe corrosion problems associated with the process (Murr and Mehta, 1982) and has low capital and operating costs (Kargi and Robinson, 1982). The sulphur can also be removed without any loss of coal (Kargi and Robinson, 1982).

DESULPHURIZATION OF COAL BY THIOBACILLUS FERROOXIDANS

Olsen et al. (1980) found that significant concentrations of sulphur could be removed from coal by Thiobacillus ferrooxidans. Approximately 77-83% of the sulphur was removed from the inoculated coal samples as compared to 5-10% in the sterile controls with 25% pulp density suspensions. The optimum pH for iron solubilization from the coals (10% pulp density) was 2.0 at 35°C. The highest rates of iron dissolution were obtained with 25% pulp density leach suspensions.

The rate of sulphur removal from coal was increased by using concentrated cell suspensions of Thiobacillus ferrooxidans and an external CO_2 and air source in a well agitated flask (Kargi, 1982). 68% of the sulphur was removed from a coal containing 11% sulphur using a concentrated cell suspension and external CO_2 source, as compared with 34% sulphur removal in experiments without a pure CO_2 supply or preconcentration of cells. A high volumetric sulphur removal rate of approximately 25 mg/lh was obtained but this was partially attributed to the high sulphur content of the coal samples used in the experiment. A sulphur removal rate based on the total external surface area of the coal particles was calculated from the volumetric rate of sulphur removal and was determined to be 5.0×10^{-3} mg S removed/cm^2h. Kargi (1982) suggested that this high surface reaction rate was due to the concentration of the cells and the external CO_2 source as both of these factors affect cell number densities on the surface of coal particles and in the solution. The high sulphur content of the coal was also implicated as a contributing factor to this rate.

DESULPHURIZATION AND SULFOLOBUS ACIDOCALDARIUS

Thiobacillus ferrooxidans has only limited use in the desulphurization of coal because it removes only pyritic sulphur (a form of inorganic sulphur) at a low rate (Kargi and Robinson, 1982). Organic sulphur removal also presents a problem in coal desulphurization. Kargi and Robinson (1982) found that Sulfolobus acidocaldarius was effective in the removal of inorganic and organic sulphur from coal. Yeast extract, a chemical oxidizing agent ($FeCl_3$), mineral salts concentration, sulphur content of coal and external surface area of coal particles affected the rate and extent of microbial sulphur removal by this microorganism.

Both yeast extract and a chemical oxidizing agent (0.01 M $FeCl_3$) decreased the rate and extent of sulphur removal. The adverse effects from the yeast were more pronounced at the higher yeast concentrations (1% as compared to 0.2%). The researchers suggested that the effect of the $FeCl_3$ may be the result of the inhibition of the microorganisms activity by the high ferric iron or chloride concentrations. In contrast, high concentrations of mineral salts increased the total amount of sulphur removed.

The total sulphur content of the two coal samples had an effect on the rate and extent of microbial sulphur removal. The volumetric sulphur removal rate for coal refuse (11% sulphur) and untreated coal (4% sulphur) was 15.6 mg S/(lh) and 3.1 mg S/(lh), respectively. These volumetric rates of sulphur removal were used to calculate sulphur removal rates based on the total external surface area of the coal particles. These rates for the coal refuse and untreated coal were $.52 \times 10^{-3}$ mg

S removed /cm^2(h) and .97 x 10^{-4} mg S removed/cm^2(h), respectively. The results showed that an increase in sulphur content by a factor of 2.8 increased the sulphur removal rate by a factor of 5.4

The rate of sulphur removal by Sulfolobus acidocaldarius increased linearly with the total external surface area of the coal at low pulp densities (15 g coal/100ml liquid). At high pulp densities, the sulphur removal rate decreased. Kargi and Robinson (1982) suggested that this may be partially due to a reduction in the solubility and transfer of gaseous nutrients (oxygen and carbon dioxide) to the microbial population or to particle build-up. The optimum pulp density for the removal of sulphur was 20% (20 g coal/100 ml liquid) under the laboratory conditions outlined in the study.

AIRLIFT FERMENTER FOR DESULPHURIZATION OF COAL OR BACTERIAL ORE LEACHING

An airlift-external recycle fermenter was constructed by Kargi and Cervoni (1983) and studies indicated that the fermenter could be used as an alternative to agitated fermenters for the microbial desulphurization of coal or for other three phase biological reactions such as bacterial ore leaching. Studies were conducted by the researchers in which the fermenter was used to remove pyritic sulphur from coal (containing 4% sulphur) using the bacterium Sulfolobus acidocaldarius. Approximately 15% of the total sulphur content of coal was removed in the first six days. After four days of lag period, nearly 30% of the total sulphur was removed.

Pyritic sulphur removal rates varied from 1.4 to 1.6 mg S pyr/lh or 0.9 to 1.0 x 10^{-4} mg S pyr/cm^2(external area of coal) h. The pH of the reaction mixture dropped from 2.5 to 2.1 during the reaction but there were no indications of adverse effects on the bacterial activity due to this drop in pH.

The airlift fermenter is easier to operate and has lower energy requirements than an agitated fermenter (Kargi and Cervoni, 1983). It eliminates potential cell desorption (from particle surfaces) and particle grinding problems that may occur in agitated fermenters. Since compressed air provides aeration and agitation in the airlift fermenter, the need for mechanical agitation is eliminated.

SULPHATE PRODUCTION WITHIN SHALE LAYERS OF COAL OVERBURDEN

Ainsworth and Blanchar (1984) studied the rate of SO_4^{2-} production with pyrite encased in its original matrix of shale and mudrock. Samples of the shale were incubated with and without inoculation by a mixed culture of Thiobacillus ferrooxidans (ATCC 23270), Thiobacillus thiooxidans (ATCC 8085) and Thiobacillus thioparus (ATCC 8158) at 298K in air and CO_2 enriched air. No significant differences in the rate of pyrite oxidation were measured with inoculated or CO_2-enriched shale samples. Ninety percent of the variation in the rate of pyrite oxidation was attributed to differences in the shale layers.

SECTION II

BIOLOGICAL REFINING OF METALS BY METHODS OTHER THAN LEACHING

SECTION II.1

ADSORPTION OF METALS

ADSORPTION OF METALS

Microorganisms can accumulate metals (biosorbents) and be used to remove and/or recover metals from aqueous solutions (Lundgren and Malouf, 1983).

Biosorption is the complexation between metal cations and negatively charged extracellular components. By definition, biosorptive complexation of metal cations does not require metabolic activity by the microorganisms and pre-exposure or adaptation to the metal is not required (Strandberg et al., 1981b).

At least two mechanisms are known to occur in microorganisms which can explain how metal biosorption occurs. The positively charged metal ions may complex with the negatively charged groups on the microorganism's surface (Strandberg et al., 1981b). Metal accumulation by this type of mechanism would be affected by competing cations (e.g. Ca^{2+} and soluble amino acids), temperature, Eh and pH (Lundgren and Malouf, 1983). Adsorption of a metal (e.g. uranium) is often not distributed evenly over all surfaces and at times, some cells within a population fail to accumulate metals (Strandberg et al., 1981a). Specific binding sites on the bacteria are not well known.

Alternatively, nucleization and crystallization may be necessary for metal biosorption. A metal deposition process has been proposed which involves the stoichiometric interaction between metal ions and the active, anionic sites on the cell walls (Beveridge, 1978). The metal that is bound to the wall then acts as a nucleation site for the growth of metal aggregates. These aggregates will grow in size until physically limited by available space. The metals are deposited on the nucleation sites in either an elemental state (eg AvIII) or hydrated state (eg. La, Ce, Fe, Pd, Ru, Er, Sm) (Beveridge, 1978). This mechanism may be responsible for uranium deposition (Beveridge, 1978).

ADSORPTION OF METALS TO BACTERIAL CELL WALLS

Cell walls of Bacillus subtilis (Marburg strain) are negatively charged and have been found to react with aqueous cations by binding them (Beveridge, 1978). This interaction "may initiate an inorganic precipitation process that allows the immobilization of substantial quantities of the soluble form" (Beveridge, 1978 p.983).

Aqueous metal ions and their hydrated complexes can act as Lewis acids in solution and interact with the wall fragments. Forty metals, including members of the alkali metals, alkaline earth metals, transition elements I,II,III and group III, IV and V elements have been analyzed for cell wall uptake (Beveridge, 1978).

Beveridge (1984) reported that significant amounts of metallic ions from an aqueous solution could bind with the bacterial cell walls of Bacillus subtilis, Bacillus licheniformis and Escherichia coli. The gram-positive cell walls of the Bacillus spp. bound more metal than the gram-negative walls of Escherichia coli; however, both the gram-negative and gram-positive walls bound quantities of metal that were greater than the number of anionic sites on the cell wall.

Bacterial cell walls expanded or contracted under the influence of specific metals. The thickness of Bacillus subtilis cell walls after treatment with indium III was 28.46 +- 2.86 nm. After treatment with conyttrium III, the cell walls were 16.33 +- 2.98 mn thick (Beveridge, 1978).

The uptake and dimensional response of walls can also be affected by time and the metal concentration. Longer incubation times generally result in thicker walls and greater metal deposition. When metals are not precipitated or crystallized (eg. U IV), long incubation periods are not necessary for rapid saturation of metals at high concentrations. For instance, Bacillus subtilis cell wall took up equal amounts (10μmol/mg dry wt. wall) of 0.1% uranyl acetate after 10 minutes and 60 minutes; however, the longer incubation period (60 min) decreased the wall dimensions (Beveridge, 1978).

IRON

ADSORPTION OF IRON ON YEAST CELLS

Iron was adsorbed on the surfaces of Lipomyces starkeyi yeast cells (Naganuma et al., 1976). Pretreatment of the cells with various metabolic inhibitors or heating did not alter the amount of iron adsorbed significantly. The major portion of the iron was released from the cell walls by washing the cells with EDTA.

Iron adsorption was pH-dependent and the optimum pH was between 3.3 and 3.6. Generally more ferric iron was adsorbed than iron in the ferrous state. The amount of iron adsorbed increased with an increase in cell and iron concentrations. The adsorption capacity of log-phase cells was the highest.

IRON-ENCRUSTED CELLS OF ACTINETOBACTER

Washed suspensions of Actinetobacter sp. caused the precipitation of iron from a suspension of colloidal ferric iron at pH 6.0 and 7.6 (MacRae and Celo, 1975). Electron microscopy showed the bacterial cells to be coated with iron particles. The coating of iron reduced the endogenous respiration and respiration rate of the cells. Endogenous respiration rates of the iron-coated cells were 32 to 72% lower than rates for non-coated cells. MacRae and Celo (1975) suggested that the inhibition of the endogenous respiration was due to the low oxygen availability or an inability of the cells to eliminate waste products through the iron barrier. The iron-encrusted cells formed large aggregates. At pH 6.0, the bacteria aggregates removed 49% of the iron from the solution and at pH 7.6, 21-52% of the iron was removed.

URANIUM

ADSORPTION OF URANIUM TO SACCHAROMYCES CEREVISIAE

Strandberg et al. (1981a) studied uranium uptake in Saccharomyces cerevisiae. The uranium accumulated extracellularly as needle-like fibrils in a layer approximately 2 µm thick surrounding the cells. Little or no uranium accumulated inside the cells indicating that uranium uptake did not involve cellular metabolism. The total capacity for uranium accumulation was 10%-15% of the original dry cell weight; however, only a portion of the cells in the population (32%) were responsible for this uptake.

Metal uptake was affected by temperature, pH and the presence of ions and amino acids. The uptake rate increased with an increase of temperature between 20 and 50°C. Initial uptake rates increased as pH increased from 2.5 to 5.5 but the maximum equilibrium distribution was obtained at pH 3.0 to 4.0. The divalent calcium cation displaced uranium from the cells. Glutamic acid and aspartic acid greatly inhibited uranium uptake by acting as uranium complexing agents which compete with the yeast cells for uranium.

The uranium was removed from the cells by chemical treatment. Nitric acid, disodium ethylenediaminetetraacetic acid and ammonium carbonate removed 59.3, 72.3 and 83.5%, respectively, of the uranium. After these chemical treatments, the initial metal-uptake rate of the recycled yeast cells was increased; however, the nitric acid and disodium ethylinediaminetetraacetic acid treatments decreased the concentration of uranium on the cells at equilibrium.

The mechanism of uranium uptake is not known but Strandberg et al. (1981a) found that uranium complexed with yeast phosphormannans and that the uranium complexation capacity of these polymers appeared to be related to their phosphate contents.

A two-step reversible kinetic model was designed by Weidemann et al. (1981) to predict the rate of adsorption of uranyl ions onto Saccharomyces cerevisiae cells. The researchers feel that the model may be useful in the design and scale-up processes for uranium recovery by the yeast.

URANIUM UPTAKE BY SACCHAROMYCES CEREVISIAE AND PSEUDOMONAS AERUGINOSA

In studies on uranium uptake by Saccharomyces cerevisiae and Pseudomonas aeruginosa, only 32% of the yeast cells and 40-45% of the bacterial cells were capable of accumulating uranium (Strandberg et al., 1981a). Investigations should be directed at the question of why only a fraction of the populations were involved in uranium uptake. If all the cells could be induced to accumulate uranium, the potential for using the cells as bioabsorbents could be enhanced.

URANIUM RECOVERY FROM AQUEOUS SYSTEMS USING STREPTOMYCES AND CHLORELLA CELLS

Nakajima et al. (1982) investigated uranium recovery from aqueous systems by immobilized Streptomyces vinidochromogenes and Chlorella regularis cells. The researchers first studied adsorption and desorption of uranium by free Streptomyces cells. The free Streptomyces cells adsorbed 96-99% of the uranium present in a 10 ppm solution at pH 6.0. The uranium adsorbed was desorbed by washing the cells with 0.1M Na_2CO_3. The same cells were reused five times in the adsorption-desorption process. However, during the recycling period, the dry weight of the cells decreased, indicating that the cells were not recycleable indefinitely.

The Streptomyces cells were immobilized by polyacrylamide gel and adsorbed 78.0% of uranium from a 200 ml uranium solution (5 ppm, pH 5).

Further studies were then conducted on Streptomyces and Chlorella cells immobilized in polyacrylamide.

Between pH 4 and 9, the amounts of uranium adsorbed by each of the species remained the same. The researchers suggested that uranium adsorptions became independent of pH after the cells were immobilized. The amount of uranium adsorbed was proportional to the temperature for both the Streptomyces and Chlorella. The uranium adsorption capacity of the cells was determined by suspending the cells in 200 ml uranium solution (20 ppm,pH6) for 4 hours for 10 cycles. The immobilized Chlorella adsorbed 159 mg/g uranium and the Streptomyces, 312 mg/g uranium on a dry weight basis.

The uranium adsorbed onto the immobilized Streptomyces cells was easily removed by washing the cells with 0.1M Na_2CO_3 solution. The cells were reused five times during which the dry weight of the immobilized cells only decreased 2% (as compared with 50% for the free cells).

EXTRACTION OF URANIUM FROM SEA WATER

Patent Specification No. 1507003 claimed that mutant blue algae (Oscillatoria) and fungi (Aspergillus niger) cultures could extract uranium from sea-water to a greater extent than the naturally occurring organisms and formed coherent colonies harvestable by filtration. The mutation is caused with x-ray radiation.

PENICILLIUM DIGITATUM AND URANIUM ACCUMULATION

Penicillium digitatum mycelium can accumulate uranium from uranyl chloride solutions (Galun et al., 1983). The basis of uranium binding may be in the wall macromolecules, cellulose, modified cellulose and particularly chitin. Killing the mycelium with boiling water or selective chemical pretreatments (alcohols, dimethyl sulfoxide or potassium hydroxide) increases the uranium uptake. This may be the result of exposing uranium binding sites either by configurational change or by removal of masking goups.

RHIZOPUS ARRHIZUS

Rhizopus arrhizus possesses a high uranium and thorium uptake capacity. Experimental evidence indicates that chitin is involved in uranium adsorption (Tsezos, 1983). In the adsorption process, uranium forms a complex with the chitin nitrogen. The uranium uptake capacity of the fungal biomass is reduced in the presence of competing ions. These ions may either compete with the uranium for the nitrogen co-ordination sites or physically block the uranium from these sites.

Rhizopus arrhizus raw biomass also had a high affinity for uranium in experiments on seawater extraction of metals (Volesky et al., 1983). A uranium sorption-desorption cycle using 20-30 mesh pellets of Rhizopus arrhizus biomass was repeated 8 times. After the first cycle, new uranium uptake was approximately 50% less than the original uranium uptake but 85% reversible in all 8 subsequent cycles. The biomass of Rhizopus oligosporus also exhibited almost total reversibility of the uranium uptake.

MOBILIZATION AND UPTAKE OF URANIUM BY ASPERGILLUS OCHRACEUS AND PENICILLIUM FUNICULOSUM

Berthelin and Munier-Lamy (1983) studied the simultaneous mobilization and uptake of uranium from different rock materials (granite, aplite, bituminous schist) by two fungi, Aspergillus ochraceus and Penicillium funiculosum.

In general, a deficiency of growth factors and soluble mineral salts in the nutrient medium that decreased the growth rate but not glucose consumption increased the uptake of uranium by fungal mycelium.

Growth of Aspergillus ochraceus was slow during incubation in deficient medium and in the presence of aplite (the uranium source) when compared to the growth in a nutrient enhanced medium. However, more uranium was immobilized by the fungal mycelium in the deficient medium than that in the nutrient enhanced medium. In the deficient medium, the uranium concentration in the mycelium was 1240 µg/g dry mycelium after 60 days incubation. Uranium uptake occurred even when growth was extremely slow. In the non-deficient medium, only 68 µg uranium/g dry mycelium was immobilized by the fungi. The pH increased from 5.7 to 8.0 in the non-deficient medium but decreased from 3.6 to 2.8 in the deficient medium.

Rock composition also played a role in buffering the medium and introducing elements that either promoted or decreased fungal growth. In the presence of granite, aplite and bitumens, the pH in the deficient medium was 5.6, 2.8 and 2.7, respectively, but in the non-deficient medium, the pH always increased from 5.0 or 5.8 to 8.5. Growth was the same in the non-deficient medium in the presence of the three different rock samples but in the deficient medium, the growth rate depended on the three rock samples. The growth rates were classified in decreasing order by rock sample as follows: granite > aplite >> bitumens.

COPPER

UPTAKE OF COPPER BY BACILLUS SUBTILIS NIGER AND PSUEDOMONAS FLUORESCENS

Baldry and Dean (1981) examined the passive uptake of copper by *Bacillus subtilis* subsp. *niger* ATCC 9372 and a strain of *Pseudomonas fluorescens*. They claimed that the copper uptake was influenced by the nutrient status of the growth media. With *Pseudomonas fluorescens*, low copper accumulations (.52-.60% dry weight) were associated with carbon, magnesium and phosphorus limitations while higher copper accumulations were associated with nitrogen, potassium and sulphur limitations. Low copper uptakes were also found when *Bacillus subtilis* subsp. *niger* grew in carbon- and magnesium-limited nutrient media. Generally, the copper uptake increased with the limiting nutrient in the order carbon < magnesium < nitrogen < potassium < phosphorous and sulphur.

COPPER EXTRACTION FROM SOLUTION AND DESULFOVIBRIO DESULFURICANS

Ilyaletdinov et al. (1977a) demonstrated that under experimental conditions, the hydrogen sulphide produced by *Desulfovibria desulfuricans* can completely remove copper from solution. In the presence of sulphate-reducing bacteria, reed and ammonia sulphate (as sources of organic matter) copper can be almost completely removed from solution. At a solution temperature of 23-25°C, the copper concentration was reduced from 1.26 mg/l to 0.01 mg/l in 9 days. At 10-13°C, the rate of copper removal was reduced and it took 15 days for the concentration to decrease to 0.01 mg/l.

LEAD

ACCUMULATION OF LEAD ON MICROBIAL CELL SURFACES

A strain of Pseudomonas fluorescens and a Citrobacter sp. accumulated lead during growth on a standard minimal medium containing lead acetate equivalent to 1000 mg/l Pb^{2+} (Aickin and Dean, 1979). The Citrobacter sp. accumulated lead equivalent to 36% of the dry weight of the organisms. This maximum lead uptake value was reached late in the logarithmic phase of growth. The lead uptake then remained constant during the stationary phase of growth but a lead salt precipitated during this period. The maximum amount of lead acccumulated by the strain of Pseudomonas was 33%. No lead salts were precipitated.

When the Pb^{2+} concentration in the medium was reduced, the amount of lead accumulated by the organisms remained between 34-40% for the Citrobacter sp. and 32-35% for the Pseudomonas sp. Lead uptake tended to be lower at lower initial lead concentrations due to the depletion of lead in the medium.

Excess amounts of glycerol 2-phosphate, the only phosphate source in the medium, was essential for maximum lead accumulations. When the concentration of glycerol 2-phosphate was reduced to a growth limiting value, the Pseudomonas sp. only accumulated 2% of the lead during growth on a medium containing 1000 mg/l Pb^{2+}.

The lead was firmly bound to the microorganisms under all experimental conditions. A sodium nitrate wash removed negligible amounts of lead during an assay procedure and subsequent washings with water removed none. The amount of lead bound to the organisms and the firmness of the binding were not affected by serial subculturing of the organisms in the presence of lead.

Resting organisms of both test strains accumulated approximately 10% lead during growth in a medium containing 1000 mg/l Pb^{2+} and a citrate buffer. Adding glycerol 2-phosphate to the culture medium of the resting cultures increased their lead uptake values to those obtained with actively growing organisms. Two enzyme inhibitors, sodium fluoride and carbonyl cyanide m-chloro-phenyl hydrazone, counteracted the effect of the glycerol 2-phosphate. Aickin and Dean (1979) suggested that the mechanism that resulted in the increased lead accumulation after the addition of the phosphate may involve an interaction between the phosphate and lead in the solution. This interaction results in the precipitation of lead phosphate ($PbHPO_4$) on the surface of the microorganisms so that it becomes very firmly bound to the surface of the bacteria. The accumulation of lead on the surface of growing organisms was assumed to occur via the same mechanism. The uptake of lead by resting organisms in the absence of a phosphate source was attributed to the interaction between Pb^{2+} and phosphate groups already attached to the cell surfaces.

To confirm that lead phosphate was the compound that precipitated on the cell walls, electron microscope studies were performed on a Citrobacter sp. grown in batch culture in the presence of lead (300 mg/l Pb^{2+}) (Aickin et al., 1979). Lead uptake was 35% of their dry weight. The cells were very opaque under transmission microscopy. The lead appeared to have formed a coating on the cell surfaces. The inhibitory action of lead on the activity of lead was negligible despite the dense deposition of lead. Elemental maps confirmed the presence of both lead and phosphate in the precipitate. X-ray microanalysis indicated that the lead was almost certainly deposited as $PbHO_4$.

ACCUMULATION OF LEAD BY MICROORGANISMS IMMOBILIZED IN POLYACRYLAMIDE GELS

A Citrobacter sp. immobilized in a polyacrylamide gel and then packed in a column removed a significant amount of lead from a solution provided an organic phosphate such as glycerol 2-phosphate was present in the solution perfusing the gel (Aickin and Dean, 1979). In the early stages of the experiment, 95% of the Pb^{2+} was removed when 200 ml of a solution containing 500 mg/l Pb^{2+} had passed through the gel. After 1000 ml of the solution had passed through the gel, 81% of the lead had been removed. The gel column remained active until 2.75 litres of the lead solution had passed through it. Approximately 52% of the lead in the 2.75 litres was removed. A lead salt was precipitated during the growth of the Citrobacter sp. in columns. This precipitate contained 66% lead.

Freshly prepared columns were used in the above experiment. Storage of the columns at 4C for one month resulted in a decrease in activity but 33% of the lead was still removed.

The researchers felt that these results warranted further work in optimizing the capability of the organisms to accumulate lead for practical purposes such as effluent control.

LEAD ACCUMULATION BY BACTERIA

Aickin and Dean (1977) examined the uptake of lead by 47 bacterial strains during growth on a medium supplemented with lead acetate equivalent to 300 mg/l Pb. Forty-two of the strains were obtained from lead polluted soil in England and Wales and were not positively identified. Five bacterial strains were obtained from culture collections. These were _Bacillus subtilis niger_ (ATCC 9372), _Pseudomonas_ fluorescens (NCIB 8906), Pseudomonas reptilovora (NRRL B334), Gulconobacter suboxydans (NRRL B72), and Klebsiella pneumoniae (NCIB 418). Their lead uptakes were 10, 8, 7, 1, and 0.1%, respectively.

Out of the total 47 bacterial strains tested, 8 strains accumulated between 32 and 36% of the lead and 27 strains accumulated more than 10% of the lead. For 37 of the bacterial strains, lead uptake amounted to more than 4% of the dry weight of the microorganisms. The lead uptake occurred in the stationary phase of the growth cycle.

Other studies were performed under identical experimental conditions which studied the uptake of cadmium sulphate and cupric sulphate. No more than 0.2% of the copper was accumulated by any of the 47 strains. The highest cadmium uptake was 1.4%. This indicated that lead was accumulated to a much greater extent than copper or cadmium. It is important to note that lead uptake also occurred when the bacteria were grown on a medium containting 0.1M citrate. The citrate acted as a buffer in the medium and chelated the lead to prevent its precipitation. The ability of microorganisms to remove lead from a lead chelate is important since chelating agents occur naturally in the environment.

FUNGI USED TO ACCUMULATE LEAD

Nine strains of fungi were also examined for their ability to accumulate lead (Aickin and Dean, 1977). Seven strains were unidentified. Of these, the highest lead uptake was 19%; the other uptake values varied between 5% and 15%. The two classified fungi Aspergillus niger (NRRL 3) and Aspergillus carbenarius (NRRL 67) accumulated 13% and 4% of the lead, respectively.

COBALT AND NICKEL

ACCUMULATION OF COBALT AND NICKEL BY SACCHAROMYCES CEREVISIAE

Fisel et al. (1978) used a strain of Saccharomyces cerevisiae suspended in a glucose-containing phosphate buffer to accumulate and concentrate cobalt (II) and nickel (II). There seemed to be a correlation between the pH of the medium and the accumulation of ions. Maximum accumulation occurred at pH 6.3. The accumulation of these metals depended on their concentration in solution. The percent accumulation decreased with an increase in the metal concentration. The results of the study indicated that this yeast could be used to concentrate cobalt and nickel from a dilute solution containing less than 5 μg/l of the two elements.

By adapting the Saccharomyces cerevisiae strain to cobalt (II), cobalt (II) purification from nickel (II) was achieved in the dilute solution.

SILVER

SILVER BIOACCUMULATION BY BACTERIAL COMMUNITIES

A mixed culture of Thiobacillus ferrooxidans and Thiobacillus thiooxidans accumulated silver during the leaching of sulphide ore minerals (Pooley, 1982). Electron microscopic examination of the bacteria indicated that the silver attached to the bacterial envelope in a granular form of the silver mineral acanthite (Ag_2S). Cleaned suspensions of the bacteria coated with acanthite were dark compared to normal suspensions. A shiny black deposit was produced upon drying at 100°C. The amount of silver accumulated per gram of dry weight varied proportionally with the silver content of the sulphide mineral leached, however, silver accumulations greater than 250 mg/g were observed.

Bacterial activity did not appear to be inhibited by the presence of the silver.

The acanthite particles were also produced artificially on the surface of the bacteria by adding soluble silver to a suspension of bacteria grown on a silver-free sulphide mineral and on elemental sulphur. Pooley (1982) concluded that sulphide ions as well as silver cations, were necessary for the formation of the acanthite particles.

A community of bacteria consisting of Pseudomonas maltophilia, Staphylococcus aureus and a coryniform organism have been found to have a great capacity for silver bioaccumulation as well as a high silver tolerance (Charley and Bull, 1979). Pseudomonas maltophilia comprised half of the population; the numbers of the other two members varied. The extent of silver removal was determined to be a function of the initial silver concentration. All silver was removed rapidly from solution at low silver concentrations (ca 12 mM). Bioaccumulations greater than 300 mg Ag per gram of dry weight biomass were observed at accumulation rates of 21 mg Ag^+ hr^{-1}(g biomass)$^{-1}$. The community showed higher rates of silver bioaccumulation than the constituent bacteria alone.

The site of actual silver deposition was not determined, but the investigators felt that the deposition could not be attributed simply to surface adsorption since the rate of silver bioaccumulation by the community decreased as the external silver concentration increased. It was hypothesized that the silver accumulation may be respiration-dependent while the respiration itself may be sensitive to silver. Any bioaccumulation would then be a compromise between these two effects.

TREATING OF WASTE PHOTOGRAPHIC PROCESSING SOLUTIONS AND SILVER RECOVERY THEREFROM USING CHEMO-SYNTHETIC SULPHUR BACTERIA

Kitajima and Abe (1979) patented a method for treating a waste fixing solution used for the fixing of silver halide photographic materials which contains at least one silver thiosulphate complex salt. $(Ag(S_2O_3)_2)^{3=}$ is thought to be the predominate salt. Chemo-synthetic bacteria belonging to the genus Thiobacillus accumulate the silver and/or complex salts into the cells under aerobic conditions. Thiobacillus thioparus, Thiobacillus novellus, Thiobacillus thiooxidans alone or in combinations may be used in the treatment process. They can be packed and stabilized into a column so that a waste solution can be passed through the column. The bacteria can accumulate silver from the solution. The bacteria can also be applied in a fixed bed in which they are immobilized on a solid substrate and a waste solution is passed through the bed. The bacteria can be in either a biologically active or inactive form. The cells are removed from the solution by sedimentation, centrifugal separation or filtration. Silver can be separated from the cells by heat treatment or by the addition of a surface active agent which destroys or solubilizes cell membranes.

CADMIUM

CADMIUM ACCUMULATION BY IMMOBILIZED CELLS OF CITROBACTER SP.

Macaskie and Dean (1984) studied cadmium accumulation by immobilized cells of a strain of Citrobacter sp. Immobilized cell columns recovered 90-96% of the metal when the cells had been pre-grown at 30°C. The immobilized cells were most efficient at removing cadmium when cadmium concentrations were 50-200 ug/ml Cd^{2+}. Metal accumulation occurred at higher and lower metal concentrations;

the upper concentration limit was 400 ug/ml before the metal accumulation ability of the cells was lost. The loaded cadmium was removed from the columns with a citrate buffer. Most of the accumulated metal was recovered in the first 500 ml of elution. The recycled columns retained 84.5% of their initial activity.

RECOVERY OF VARIOUS METALS FROM SOLUTION

MICROORGANISMS AS BIO-FILTERS

Brierley and Brierley (1980) stated that "it is foreseeable that living organisms will be used as 'bio-filters' to recover metals from solutions too dilute in metal concentrations to economically use other processes for metal recovery."

Brierley and Brierley (1980) studied the biological and chemical agents which participated in the removal of selenium, molybdenum and uranium from uranium mine waste water passing through a treatment pond system. The treatment pond system was effective in lowering the concentrations of selenium, molybdenum and particularly uranium (4.6 and 5.8 ppm uranium was reduced to 0.8 ppm). Large populations of microorganisms present in the water pumped from the uranium mines were suspected of participating in the removal of the elements from water. These included Desulfovibris and/or Desulfotomaculum, Thiobacillus thiooxidans, Thiobacillus thioparus and Thiobacillus denitrificans.

Laboratory experiments indicated that the sulphate-reducing bacteria may have an important role in the removal of uranium and selenium from solution. The mechanism involved in the removal of these metals was not known. Brierley and Brierley (1980) suggested that sulphide production may be important as molybdenum may be precipitated as a sulphide. Soluble hexavalent uranium may be converted to an insoluble quadrivalent form in the reducing conditions.

RECOVERY OF METALS FROM A BACTERIAL LEACHING SOLUTION WITH SULPHATE-REDUCING BACTERIA

A continuous culture of sulphate-reducing bacteria was used to recover copper, zinc and iron from a bacterial leaching solution (Tomizuka and Yagisawa, 1978). The optimum pH for growth and for metal removal was 6.0. Maximum rate of metal removal was obtained at 40% metal concentration of the original leach solution. Maximum rate of removal of copper, zinc and iron was 47.5 mg/l/hr., 18.1 mg/l/hr and 25.7 mg/l/hr, respectively. A black precipitate formed which contained 19.96% Cu, 6.13% Zn,and 10.95% Fe.

METAL UPTAKE BY PENICILLIUM OCHRO-CHLORON

Penicillium ochro-chloron could be used to concentrate and recover metals from substrates with low metal concentrations. A strain of this fungus has been found to tolerate various concentrations of zinc, manganese, iron, lead and cadmium but has shown sensitivity to mercury, nickel and cobalt (Okamoto et al., 1977). Studies on copper accumulation on Penicillium ochro-chloron have indicated that the highest bioaccumulation occurs in solutions with low metal concentrations (Stokes and Lindsay, 1979). The uptake is rapid (1-7 days) and is best in static conditions when the fungus forms a mat (Stokes and Lindsay, 1979). This mat is easily removed for harvesting. The fungus grows rapidly in darkness or light and over a wide pH range.

The mechanism of tolerance to the heavy metals is not known. Reduction in the permeability of the cell to toxic metals may be important (Okamoto et al., 1977). Studies by Okamoto and associates (1977) indicated that Penicillium ochro-chloron possesses the ability to control the quantity of copper and zinc uptake. Cellular contents of these metals were kept constant at higher concentrations in the surrounding solutions; this was also exhibited to some extent with cadmium. This enabled the fungus to grow with high metal concentrations. However, the fungus could not regulate nickel or cobalt uptake and as a result, it could not survive at the higher concentrations.

A reduction in the potassium and magnesium content occurred in the cells at high concentrations of copper, zinc and, frequently cadmium. This reduction closely corresponded to the period when cellular levels of copper, zinc and cadmium were constant. A regulation mechanism involving these cations may be involved; however, a reduction in potassium and manganese content was not accompanied by an increase in the cellular content of copper, zinc and cadmium at their lower concentrations. It is therefore difficult to speculate on the importance of an exchange in metal toxicity.

FUNGI AS METAL ADSORPTION AGENTS

Nemec et al. (1977) patented a process for treating the mycelia of Penicillium aspergillus to produce a stiffened, water-insoluble, granulated product for the retention of metal ions such as uranium, radium and lead from aqueous solutions. The mycelia were stiffened by adding polymerizable components to them (formaldehyde, resorcinol, urea, m-phenyldiamine, gelatine, starch, bone glue, casein, epoxide resin and acrylic resin). The sorbent capacity of the mycelia for uranium after this stiffening process ranged between 39 and 102.5 mg/g.

Patent Specification No. 1514612 also describes a method for reinforcing mycelia of fungi which are suitable for the retention of uranium, radium and lead. Penicillium chrysogenum, Aspergillus niger, Aspergillus ochraceus, Streptomyces aureofaciens, or Mycelium sterilium may be used in this process. The mycelium of the fungus is brought into contact with polymerisable components (formaldehyde,

formaldehyde and resorcinol, formaldehyde and urea, polvinyl acetate emulsion) as well as with a surface-active agent (diethanolamide of a higher fatty acid) while under agitation in a liquid dispersion medium. Under suitable conditions, solid granules of the reinforced mycelium will form. Sorptive capacities of the reinforced mycelium for uranium range from 90 to 98.6 mg/g.

Drobot (1983) patented a simple, relatively low cost process for separating low concentrations of dissolved salts of various metals from aqueous media by treatment with fungi. The process recovers precious metals such as platimum, rhodium, palladium, ruthenium, iridium, gold and silver, as well as base metals, such as zinc, aluminum, iron, copper, tin and nickel. The method is useful for recovering these metals from acidic solutions (pH < 4).

A metal is recovered from an aqueous solution containing the metal in a soluble form by inoculating or contacting the solution with a live fungus for 4 hours to 6 days. The fungus converts the soluble metal into the insoluble form and the metal is deposited and concentrated in the biological mass. Fungi that may be used in this process include Cladosporium, Penicillium, Trichoderma, Black Mycelium, Aureobasidium and combinations of two or more of the aforementioned fungi.

The metals may be separated from the fungal biomass by filtration or centrifugation. Magnetic or electromagnetic separation of the fungal concentrates may also be used. Specific examples of metal removal from aqueous solution by the fungi are given in the patent.

SECTION II.2

EXOPOLYSACCHARIDES AS ADSORPTION AGENTS

EXOPOLYSACCHARIDES AS ADSORPTION AGENTS

Organisms excrete negatively charged polysaccharides which form a matrix around the cells. The polysaccharides are suitable substances for the attachment of metal cations.

SURFACE ACTIVE POLYMERS AND METAL IONS

Primary film-forming marine bacteria such as Pseudomonas atlantica and Pseudomonas spp. produce surface active polymers that form insoluble precipitates with the soluble salts of iron, copper, and lead (Corpe, 1974). The polymers formed water-insoluble precipitates with cationic detergents and soluble salts of iron, copper and lead. The polymers were slightly different with regard to metal affinity. The neutral polymer solutions formed nondiffusible complexes with cobalt, nickel, zinc, calcium and magnesium but no special selectivity for a particular metal was observed.

EMULSAN, AN EXTRACELLULAR BIOEMULSIFIER

Emulsan, an extracellular bioemulsifier of Actinobacter calcoaceticus RAG-1, and emulsanosol, a cream consisting of small hydrocarbon droplets coated with water and emulsan, can bind uranium over a wide range of initial uranyl ion concentrations (Zosim et al., 1983). Under the conditions defined in an experiment performed by Zosim et al. (1983), the binding capacity of emulsan for uranium was 0.9 µm bound uranium per milligram of emulsan. Much larger amounts of uranium were bound by emulsan associated with hexadecane-water interfaces (emulsanosols) than by free emulsan in an aqueous solution. By using emulsanosols, more than 3.5 µm uranyl ions were bound per milligram of emulsan. Following uranium binding, more than 90% of the bound uranium was resorbed for recovery purposes and the emulsanosols were recycled. Experimentation indicated that the presence of other ions in an aqueous solution may interfere with the metal ion adsorption by emulsan and inhibit the adsorption of the valuable metal.

Monovalent and divalent metal ions competed with the uranyl ion for binding by emulsan resulting in a decrease in the amount of uranium bound in the presence of these ions.

SECTION II.3

MICROBIAL CHELATES

MICROBIAL CHELATES

A number of metal chelates can be synthesized by microorganisms. Zajic (1969b) grouped these metal-binding compounds as:

1. amino acid chelates
2. polypeptide or protein chelates
3. metallophenolic complexes
4. metallophosphate complexes
5. organic acid chelates
6. polyhydroxy compounds
7. metalloporphyrins

A complete summary of specific metal chelates is given by Zajic (1969b).

A CHELATING POLYMER FROM ASPERGILLUS NIGER

A chitosan-glucan complex obtained from *Aspergillus niger* mycelium wastes (from fermentations for the production of citric acid) was proposed as a chelating polymer with a high capacity for removing cupric and mercuric ions from aqueous solutions (Muzzarelli et al., 1980). The chitosan-glucan of any mesh size is packed into columns which can be regenerated with solutions of potassium cyanide or with mixtures of sulphuric acid and ammonium sulphate. The efficiency of ion removal increased with temperature and mesh size number.

SECTION II.4

FLOCCULATION OF METALS

FLOCCULATION OF METALS WITH ZOOGLOEA RAMIGARA

Norberg and Persson (1984) recently described a process involving the batchwise flocculation of copper, cadmium and uranyl ion solutions by adding Zoogloea ramigara biomass. The biomass was added to a heavy metal solution at an appropriate pH value that supported floc formation. Metal uptake was rapid (within 15 minutes). Adjustment of the pH produced selective metal uptake. Uranium was adsorbed to the biomass at approximately pH 3.5, copper at pH 5.5 and cadmium at pH 6.5. Other ions interacted with uranium, copper and cadmium adsorption by the biomass and inhibited adsorption of these valuable metals. For example, trivalent aluminum, divalent manganese and monovalent sodium decreased the copper uptake capacity of the biomass. The flocs formed were large (2-8mm), rigid and porous, and rapidly separated by centrifugation. The resulting pellets of bacterial biomass and adsorbed ions were diluted with an equal volume of water and mechanically broken down into a slurry which was acidified with hydrochloric acid and equilibrated for 15 minutes. This process resulted in the release of the metals from the biomass. The metal binding capacity of the regenerated biomass was not affected by the acid treatment. The bacterial flocs and dissolved metal ions were separated by centrifugation. The regenerated biomass was added to a reservoir containing fresh biomass and stirred to insure a homogeneous mixture. The biomass was then ready for reuse.

SECTION II.5

INTRACELLULAR UPTAKE OF METALS

INTRACELLULAR UPTAKE OF METALS

INTRACELLULAR UPTAKE OF CADMIUM BY PSEUDOMONAS AERUGINOSA

Cadmium uptake by a cadmium chloride ($CdCl_2$) -tolerant bacterium, Pseudomonas aeruginosa G-1, has been investigated (Horitsu et. al., 1979). Levels of up to 800 ppm cadmium and 180 ppm cobalt were tolerated by the bacterium. It did not tolerate any levels of mercury (Hg^{2+}) or chromium (Cr^{6+}).

Upon chemical analysis of the cells, 12% of the cadmium was found in an insoluble polyphosphate-polysaccharide fraction; 88% was found in the protein and nucleic acid fraction of the cytoplasm. A slight morphological change was observed with cells grown with cadmium when compared with cells of the control. These cells lacked the sticky materials adhering to cells that were grown in the absence of cadmium.

INTRACELLULAR UPTAKE OF URANIUM BY PSEUDOMONAS AERUGINOSA

Uranium uptake by Pseudomonas aeruginosa was studied by Strandberg et al. (1981a). The uptake of uranyl ions was extremely rapid. The uranium associated with the cells within 5 to 10 seconds after the initial cellular contact with uranium. The metal formed dense intracellular deposits indicating the possible involvement of metabolically mediated active transport. The total capacity for accumulation by Pseudomonas aeruginosa was 10 to 15% of the original dry cell weight; however, only 44% of the bacterial cells were responsible for this uptake. Environmental factors such as pH, temperature and the presence of ions did not affect the uranium accumulation by this bacterium.

INTRACELLULAR UPTAKE OF MERCURY BY A MERCURIC CHLORIDE-TOLERANT BACTERIUM

Horitsu et al. (1978) isolated a mercuric chloride-tolerant bacterium from activated sludge. The bacterium, Pseudomonas oleovorans, is a motile, gram negative rod, 0.9 µm x2.0 µm in size. It is tolerant of metal ion concentrations up to 350 ppm Hg^{2+}, 100 ppm Cd^{2+}, 40 ppm Cr^{6+}, and 1000 ppm Cu^{2+}. The cells growing with mercury had a less rigid cell surface and electron-dense granules in the cytoplasm when compared with cells growing in a mercury-free environment. Approximately 80% of the mercury taken up by the resting cells occurred in the cytoplasm and 20% in the cell envelope fraction. The mercury was concentrated in the nuclear acid fraction of the supernatant fluid. Mercury was found in the insoluble polyphosphate-polysaccharide, mucopeptide and lipopolysaccharide-polysaccharide fractions. Approximately 20% of the mercury taken up by growing cells accumulated in the cytoplasm and 80% in the insoluble fraction.

SECTION II.6

PRECIPITATION OF METALS

PRECIPITATION OF GOLD BY FUNGI

Pol'kin et al. (1975) claimed that investigations have been carried out on the process of gold precipitation from hydrochloric acid solutions by means of mould fungi. The fungi Aspergillus was most efficient as a precipitating agent.

The highest precipitation rate was obtained with 105-day-old fungi. When gold was in a solution at a concentration of 500 mg/l with 5 g/l HCl, 99% of the metal was precipitated as acidity increased, the extent of the precipitation of gold decreased. With an HCl concentration of 200 g/l, precipitation of gold did not exceed 30%. When previously dried fungus culture at 150-200°C was utilized, the amount of gold extracted increased by 10-12%. An investigation into the biochemical composition of gold-adsorbing fungi revealed that the chemical activity of some gold precipitators (protamines and globulines) increased after a moderate dehydration or thermo-acid tie.

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